Influence of Magnetic Fields and their Characteristics on cortical Excitability.

Study on healthy adults and pathological aging.

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AUTORIZAN

La defensa del presente trabajo de Tesis Doctoral titulado "Influence of Magnetic Fields and their Characteristics on cortical Excitability. *Study on healthy adults and pathological aging.*", que Dña. Paula Davila Pérez ha realizado bajo nuestra supervisión y que presenta las condiciones necesarias de originalidad y rigor científico para optar al título de Doctora con Mención Internacional por la Universidad de A Coruña.

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A mis padres y mi hermano,
por su apoyo y su cariño constante.
Y a David, por estar siempre a mi lado.

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Abstract

This doctoral thesis intended to answer two questions in relation to the interaction between dynamic and static magnetic fields, and the motor cortex.

During the first two experiments, we studied factors that influence the reproducibility of transcranial magnetic stimulation (TMS). These investigations have shown that two types of relevant factors impact the effects and the reproducibility of the TMS: (1) technical or modifiable factors, in particular the TMS pulse waveform and current direction; and (2) physiological factors, specifically the influence of physiological aging and age-related diseases, such as Dementia due to Alzheimer's disease and type 2 Diabetes Mellitus.

In a second type of study, a reduction of motor cortex excitability and reactivity was observed after applying transcranial static magnetic stimulation (tSMS). However, this reduction was only captured by a specific type of TMS waveform, indicating that the action of the tSMS is centered on specific cortical interneuron networks. Electroencephalographic recordings quantified an increase in the range of beta frequencies in fronto-central regions, this increase was negatively correlated with the reduction in cortical excitability.

Resumo

Esta tese de doutoramento pretende responder dúas cuestións relacionadas coa interacción dos campos magnéticos dinámicos e estáticos co córtex motor.

Nos dous primeiros experimentos estudáronse factores que inflúen na reproducibilidade da estimulación magnética transcraneal (TMS). Estas investigacións demostraron que tanto os

efectos como a reproducibilidade da TMS vense afectados por dous tipos de factores relevantes: (1) factores técnicos ou modificables, en particular a forma de onda e a dirección da corrente dos pulsos da TMS; e (2) factores fisiolóxicos, en concreto a influencia do envellecemento fisiolóxico e as enfermidades relacionadas co envellecemento, coma a demencia por enfermidade de Alzheimer e a Diabetes Mellitus tipo 2.

Nun segundo tipo de estudo observouse que a estimulación magnética estática transcraneal (tSMS) produce unha redución da excitabilidade e reactividade do sistema motor. Porén, esta redución só puido ser capturada por un tipo específico de forma de onda de TMS, o que indica que a acción da tSMS céntrase en redes interneurais específicas. Nos rexistros electroencefalográficos cuantificouse un aumento das frecuencias do rango beta nas rexións fronto-centrais, que está inversamente correlacionado coa diminución da excitabilidade.

Resumen

Esta tesis doctoral pretende responder dos cuestiones relacionadas con la interacción de los campos magnéticos dinámicos y estáticos con el córtex motor.

En los dos primeros experimentos se estudiaron factores que influyen en la reproducibilidad de la estimulación magnética transcraneal (TMS). Estas investigaciones demostraron que los efectos y la reproducibilidad de la TMS se ven afectados por dos tipos de factores relevantes: (1) factores técnicos o modificables, en particular la forma de onda y dirección de corriente de los pulsos de la TMS; y (2) factores fisiológicos, en concreto la influencia del envejecimiento fisiológico y de enfermedades relacionadas con el envejecimiento, como la demencia por enfermedad de Alzheimer y la Diabetes Mellitus tipo 2.

En un segundo tipo de estudio se observó que la estimulación magnética estática transcraneal (tSMS) produce una reducción de la excitabilidad y reactividad del sistema motor. Sin embargo, esta reducción sólo se pudo captar por un tipo específico de forma de onda de TMS, indicando que la acción de la tSMS se centra sobre redes interneurales específicas. En los registros electroencefalográficos se cuantificó un aumento de las frecuencias del rango beta en regiones fronto-centrales, que está inversamente correlacionado con la disminución de la excitabilidad.

List of Abbreviations

AD	Dementia due to Alzheimer's
AMT	Active motor threshold
AP	Anterior-posterior
APOE	Apolipoprotein-E
AUC	Area under-the-curve
BDNF	Brain-derived neurotrophic factor
bi_{AP-PA}	Biphasic anterior-posterior—posterior-anterior
bi_{PA-AP}	Biphasic posterior-anterior—anterior-posterior
CBI	Cerebello-brain inhibition
CDR	Clinical Dementia Rating
CNS	Central nervous system
CP	Conditioning pulse
cSP	Cortical silent period
cTBS	Continuous theta-burst stimulation
D-wave	Direct wave
DBS	Deep Brain Stimulation
DMF	Dynamic magnetic fields
EC	Eyes closed
EEG	Electroencephalography
EMG	Electromyography
EO	Eyes open
EOG	Electrooculography

EPSP	Excitatory post-synaptic potentials
FDI	First dorsal interosseous
FDR	False discovery rate
FWER	Familywise error rate
GABA	gamma-Aminobutyric acid
HSD	Honestly significant difference
I-waves	Indirect waves
ICA	Independent component analysis
ICC	Intra-class Correlation Coefficient
ICF	Intracortical facilitation
IFCN	International Federation of Clinical Neurophysiology
IPSP	Inhibitory post-synaptic potentials
ISI	Interstimulus Interval
iTBS	Intermittent theta-burst stimulation
LAI	Long afferent inhibition
LFMS	Low Field Magnetic Stimulation
LICI	Long-interval intracortical inhibition
LTD	Long-term depression
LTP	Long-term potentiation
Max+	Maximum facilitation
Me-ANOVA	Mixed-effects analyses of variance
Me-OLR	Mixed-effects ordered logistic regression
MEP	Motor evoked potential
MMSE	Mini-mental status examination
$mono_{AP}$	Monophasic anterior-posterior

$mono_{PA}$	Monophasic posterior-anterior
MRI	Magnetic Resonance Imaging
MSO	Maximal stimulator output
NHPT	Nine-hole peg test
NIBS	Non-invasive Brain Stimulation
NSE	neuron-specific enolase
nTMS	Neuronavigated Transcranial Magnetic Stimulation
PA	Posterior-anterior
PTN	Pyramidal tract neuron
RMT	Resting motor threshold
rs-EEG	Resting state electroencephalography
rTMS	Repetitive Transcranial Magnetic Stimulation
SAI	Short afferent inhibition
SICI	Short-interval intracortical inhibition
SMF	Static magnetic fields
T2DM	Type-2 Diabetes
tACS	Transcranial Alternating Current Stimulation
TBS	Theta-burst stimulation
tDCS	Transcranial Direct Current Stimulation
TES	Transcranial Electric Stimulation
TMS	Transcranial Magnetic Stimulation
TP	Test pulse
tRNS	Transcranial Random Noise Stimulation
tSMS	Transcranial Static Magnetic Stimulation
VNS	Vagal Nerve Stimulation

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PREFACE: INTRODUCTION, HYPOTHESIS AND OBJECTIVES

1 General description and importance of Non-invasive brain stimulation

Over the course of history, humans have been inquisitively curious about electrical and magnetic phenomena around them. How electric and magnetic forces influence the physiology of different biological systems has consequently become a fundamental question with proven relevance for medical practice. Widely known examples of the use of electrical fields for the diagnosis and treatment of several disorders are techniques as common in medical practice as electrocardiography or electroconvulsive therapy.

During the last decades, this interest has increasingly expanded the application of electrical and magnetic fields on humans for clinical and research purposes, both through invasive (involving intra-cranial implanted electrodes) and non-invasive methods of administration. Unlike invasive techniques such as deep brain stimulation (DBS) or vagal nerve stimulation (VNS), non-invasive brain stimulation (NIBS) proceedings do not need surgery or other interventions in order to reach brain structures. Therefore, and not surprisingly, the use of magnetic and electrical fields for medical purposes as part of the so-called NIBS techniques is a growing scientific topic that has become a matter of deep interest for the scientific community. Nowadays, many laboratories and clinics use NIBS methods as a research, diagnostic, or therapeutic tools. NIBS techniques allow to assess and change brain cortical reactivity, excitability, and plasticity processes as well

as modulate different brain states. The combination of NIBS with common neurophysiological recording tools such as electromyography (EMG), electroencephalography (EEG) or magnetic resonance imaging (MRI) allow to assess changes in the nervous system in a quantifiable and objective way.

Notable examples of NIBS are transcranial direct current stimulation (tDCS) that uses electrical currents to modulate the central nervous system (CNS); or transcranial magnetic stimulation (TMS) where time-varying magnetic fields are able to both modulate and stimulate nervous system structures.

TMS was first described by Barker and colleagues (Barker, Jalinous, & Freeston, 1985) over 30 years ago and since then it has become a powerful diagnostic and treatment tool. TMS has been used to investigate cortical reactivity and excitability (within a cortical area or the interaction between different cortical areas), study brain behavior, and assess neurophysiology of healthy brain and neuropsychiatric disorders. In addition, when applied as a train of repeated pulses with a certain frequency, thus termed repetitive TMS (rTMS), modulates the activity of neural networks lasting beyond the stimulation time. The treatment of drug-resistant depression with rTMS is a paradigmatic example of the medical use of TMS, approved by FDA (FDA approval K061053) in 2008 and widely used across the world. Besides, FDA approved the use of TMS for presurgical motor and language mapping as a diagnostic tool. Ever since, many other protocols and applications for different neuropsychiatric diseases have been characterized.

More recently, new NIBS techniques like transcranial static magnetic stimulation (tSMS), have been described and shown to change cortical reactivity and excitability on humans (Oliviero et al., 2011) in a painless, reversible and safe way (Oliviero et al., 2015). Unlike TMS, tSMS uses non-time varying magnetic fields in order to modulate cortical areas.

Despite the growth and the widespread interest, there is still an important lack of deep understanding of the underlying mechanisms of NIBS techniques and their interaction with the neural substrate. This is especially relevant for newly reported ways of modulating the brain like

tSMS. Although tSMS research field is moving fast both in terms of animal and human experiments, there are still plenty of questions to be answered in order to begin to understand how static magnetic fields (SMF) interact with the brain. Additionally, to allow the finding of meaningful changes in cortical reactivity or excitability following an intervention, the scientific community requires reliable neurophysiological evaluations. Several factors may influence the response to NIBS methods such as TMS, thereby reducing its trial-to-trial reproducibility and influencing any possible outcomes.

The principal intention of the present thesis is to deepen on the knowledge of the NIBS interaction with cortical areas and components by answering two main questions.

First, we aimed to better understand potentially important elements influencing the reliability of TMS, which is world-wide known and the most used NIBS technique. Despite its relevance, TMS has a considerable trial-to-trial variability. Determining the influence of different factors may help improving the reproducibility and hence the utility of the technique both for diagnosis and treatment purposes. We investigated two main groups of factors that we considered important and yet to be fully understood. Based on current theoretical models, technical parameters, for instance TMS different waveforms and current directions activate and interact with different neural components. Therefore, we hypothesized they may play an important role on the reproducibility of different TMS protocols that might be driven by specific neural components and/or connections. The second group of factors we investigated were physiological and pathophysiological factors that have potentially a great effect on brain reactivity and plasticity processes, such as age and age-related diseases (i.e. Dementia due to Alzheimer's disease, AD, and one of the most common metabolic disorders with increasing prevalence related to age, type-2 diabetes mellitus, T2DM). Therefore, we proposed the previous factors will change TMS-cortex interactions as well as they will have a considerable impact on the reliability of TMS.

Second, we intended to further investigate the behavior and changes on excitability of motor cortical areas after the exposure to tSMS, a novel and promising NIBS technique. To this

effect, we used TMS and EEG as neurophysiology evaluation tools in order to record brain reactivity changes that last beyond the time of stimulation. As previously mentioned, specific TMS physical parameters may interact with different neural components. We hypothesized that tSMS may not equally influence the diverse cortical components. Therefore, as a first goal we used different TMS parameters so as to elucidate whether the variations on cortical excitability after tSMS are due to the distinct activation or deactivation of particular cortical elements inspected by a specific TMS waveform or current direction. A second goal of this study was to evaluate the modulation of motor cortex excitatory/inhibitory balance after the exposure to tSMS by using specific TMS paradigms that help us to better understand the functioning of inhibitory and facilitatory brain circuitry. Moreover, EEG successfully examines electrical changes of the convexity of the brain across time in a very precise way. We propose that tSMS will not only change cortical reactivity but also brain spontaneous oscillatory activity, as measured by EEG. Thus, EEG recordings together with TMS assessments can help us to better comprehend physiological changes due to tSMS.

NON-INVASIVE BRAIN STIMULATION TECHNIQUES (NIBS): STATE OF THE ART.

NIBS is a heterogenic group of tools that use electric currents and/or magnetic forces to modulate the brain. However, NIBS techniques face a fundamental issue given that they need to go through anatomical "barriers", including scalp, skull, meninges and cerebrospinal fluid, until they reach the cortical layers. The most commonly used NIBS techniques ultimately utilize the electric properties of the neural components, although they can be divided in two larger groups depending on the means by which they reach cortical layers: (1) techniques that use magnetic fields to painlessly go through the scalp and reach the brain cortex, such as TMS or low field magnetic stimulation (LFMS); and (2) techniques that use electrical fields. In this second group, we can include transcranial electric stimulation (TES), tDCS, transcranial alternating current stimulation (tACS) or transcranial random noise stimulation (tRNS). Recently, a novel method that cannot be classified into any of the two larger groups depicted above, has been described in healthy humans (Oliviero et al., 2011). This new NIBS technique uses SMFs, hence it does not induce electric fields, to decrease cortical excitability beyond the time of stimulation. The mechanisms by which the SMFs reduce cortical reactivity and excitability have not been well elucidated yet.

TES, which uses high voltage electric currents in order to stimulate the cortex, was a pioneer method on the brain stimulation field but it is painful and therefore less suitable for the stimulation of an awake and conscious human brain. In contrast to the rest of the NIBS techniques

described in this manuscript, when TES is applied to motor cortex it has been shown that its high voltage electric currents activate the corticospinal tract mainly at a subcortical level partially bypassing the cortex. tCS procedures (i.e. tDCS, tACS and tRNS) solved the disadvantage of the high discomfort by using lower intensities of stimulation. The lesser amount of energy used in tCS is able to modulate the cortex, namely, is able to either depolarize or hyperpolarize a certain area, but the depolarization is not sufficient to initiate and action potential. In other words, if applied to the motor cortex, tCS techniques cannot elicit registrable responses in the muscles. With the arrival of technological development, magnetic fields could be used to pass through the anatomical “barriers” and reach the brain creating a suprathreshold current that activates the cortical with little to no discomfort, as in the case of TMS.

For the purposes of the present work we will summarize and deepen on the current knowledge and advances of two specific NIBS techniques that were employed during the experiments of the thesis, TMS and the novel tSMS.

2 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation is the most commonly used technique among the NIBS. This technique not only modulates the brain cortical activity but also activates neural circuits. This fact makes TMS a great tool for the *in vivo* evaluation of brain physiology of health and disease. At a research level, this means that we can explore more about how the brain works in a painless and safe way. This includes different neurophysiological processes such as brain reactivity, connectivity or cortical plasticity. At a clinical level, it allows us to use it as a powerful diagnostic (Groppa et al., 2012) and treatment tool (Lefaucheur et al., 2014).

TMS can be applied using one stimulus at a time (single-pulse TMS), a pair of stimuli separated by a given time interval (paired-pulse TMS), or using trains of stimuli with an internal

frequency (rTMS). Furthermore, in combination with imaging techniques such as EEG or MRI, we can investigate TMS-brain interactions and relationships with behavior and cognitive processes in more depth. The different TMS protocols and techniques in combination are described in *Methodology* (*Chapters 4 – 6*).

During the following sections of the present chapter we will review the fundamental physics and characteristics of TMS as well as the common industrial presentations (devices and coils). Furthermore, the outcome and effects of TMS depend on the interaction between the physical properties of the TMS equipment, the TMS stimulation parameters as well as the physiological factors of the target brain tissue. We will describe the most relevant biophysical and physiological factors that might influence TMS-brain interactions and the background of theoretical canonical models of neural component activation, that are key for the present work. Finally, when dealing with a medical device, reproducibility and safety should always be taken into consideration. Hence, as part of this background summary, we will review the latest reports on TMS test-retest reliability and discuss the main TMS safety topics.

2.1 Fundamentals of Transcranial Magnetic Stimulation

Transcranial Magnetic Stimulation (TMS) is a powerful technique that utilizes the properties of magnetism to effectively stimulate the human brain. A TMS pulse consists of a rapidly changing electrical current sent through the wiring of a coil, we will call this the primary current or field. In the coil, the generated primary current fluctuates in a very short period of time (<1 millisecond (ms)). As we know from Faraday's principles of electro-magnetism induction, these fast time-varying electrical currents are able to induce magnetic fields. Coil-induced magnetic fields last for about 100 µs and can reach up to 2-2.5 Tesla (T) with a flux perpendicular to the plane of the coil and the primary current. In turn, if the coil is placed tangential to the scalp, when the magnetic field reaches tissues with electrical properties, such as the brain cortex, a

second electric current is induced parallel to the original electric current but in opposite direction (Hallett, 2007). Hence, this second electric current runs parallel to the coil, original current and also to the cortex (see ***Figure 2.1*** for a schematic representation). Ultimately this current originated within cortical neural components initiates an action potential and therefore, activates the pathway (Wagner, Valero-Cabré, & Pascual-Leone, 2007).

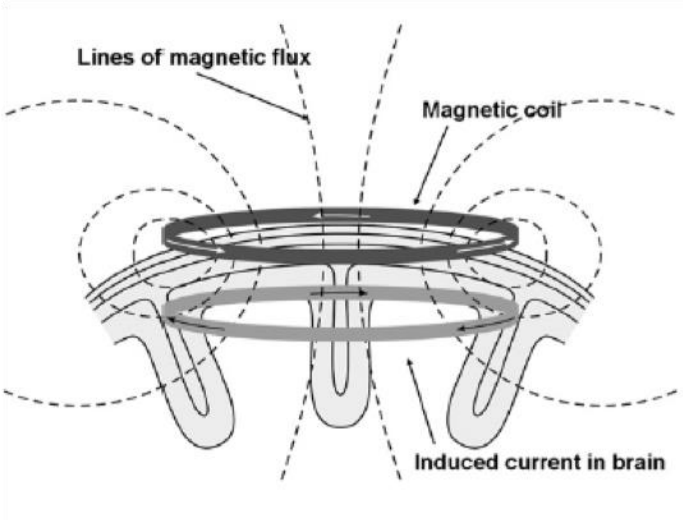

Figure 2.1. Representation of the primary electric current in the coil, the generated magnetic forces and the electric current induced in the brain surface.
From Hallett, 2007.

At a cellular level, TMS activates neural components at their axons and dendritic trees rather than at their cell bodies, where the excitation threshold is much higher. Axons and dendritic trees are the cell parts with the highest ion density, but their activation highly depends on their orientation in relation to the electric current and the magnetic flux. In early studies on *in vitro* and *in vivo* nerves (Maccabee, Amassian, Eberle, & Cracco, 1993; Maccabee et al., 1998) showed that axons are preferentially activated when running parallel to the current. If the currents are not totally parallel, then the most effective place is where the axons bend. This phenomenon can be explained by Faraday's and Lenz's physical laws of electromagnetic induction in which the second

electric field is greater in parallel electro-sensitive materials and depends on the electromotive force generated by a rapidly changing magnetic flux.

In terms of cell populations, previous studies have suggested that different TMS parameters such as waveform or current direction, may activate different neural components of various cortical layers. This means that the TMS electric currents running parallel to the cortex, as well as their directionality are of a great relevance to understand where and how the neural activation takes place.

Early studies on animal models (Amassian, Stewart, Quirk, & Rosenthal, 1987; Patton & Amassian, 1954), recorded the activation of the corticospinal tract after electric stimuli (i.e. TES) over the primary motor cortex by using epidural electrodes over the spinal cord. These epidural recordings revealed the existence of a high frequency (approximately 600Hz) complex responses. Within the complex response, two distinct groups of waves can be distinguished based on their endurance to cortical cooling or cortical ablation. First, D-wave or direct wave that survives cortical removal or depression after cooling, and therefore translates the direct activation of the pyramidal tract neuron (PTN) at an axonal level. Second, I-waves or indirect waves, a complex group of smaller waves that appear at later latencies with the same internal discharge rate of about 600Hz (approximately 1.5 ms between waves) (see diagram of primary motor cortex and D- and I-waves in ***Figure 2.2***). In order to explain their occurrence, two theoretical models emerged. The first model, based on cell membrane oscillatory properties, proposes that I-waves are explained by the activation and reverberation of high-frequency excitatory neural circuits in the cortex that end up with the stimulation of the PTN (Amassian et al., 1987). Data from later studies (Day et al., 1989; Sakai et al., 1997) offered a second and, most probably, complementary theoretical frame where the main assumption is that motor cortex transcranial stimulation activates different subsets of neural components in different cortical layers. This second hypothesis proposes that I-waves decode the activation of a network or chain of interneurons that generate synchronous volleys of excitatory and inhibitory post-synaptic potentials (EPSP and IPSP) that will finally evoke a PTN

response. The first animal-model studies distinguished two groups of I-waves depending on their differential depression during cooling of the motor cortex: (1) An early I-wave (the I1-wave), that appears about 1.5 ms after the D-wave and persist longer during cooling, and (2) subsequent I-waves (late I-waves) that are soon affected by the cold. I1-wave is thought to represent the indirect monosynaptic activation of the PTN through excitatory interneurons in layers II and III. While the late I-waves have been shown to be more easily influenced by processes that change cortical excitability and studies have argued that they may reflect the activation of horizontal cortico-cortical connections in those same cortical layers, that originate from surrounding cortical regions or perhaps other brain structures (Di Lazzaro & Ziemann, 2013; Cirillo & Perez, 2015). In summary, early and late I-waves translate less or more complex activation of intracortical neural circuits that will end up evoking a PTN response.

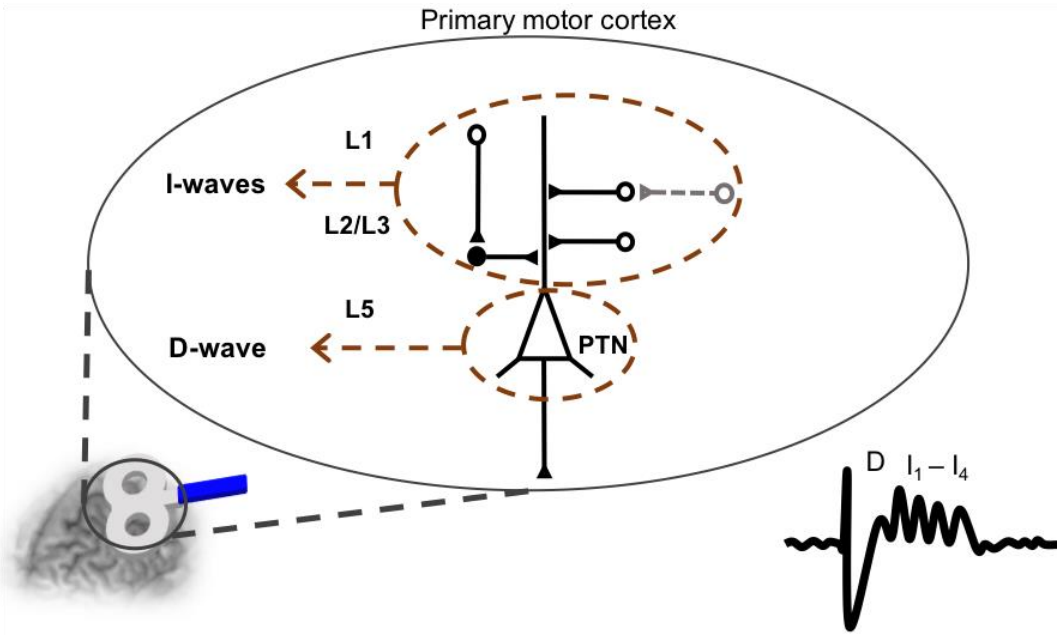

Figure 2.2. Schematic representation of the neural components that generate the direct wave (D-wave) and the indirect waves (I-waves).

Abbreviations: D- wave, direct wave; I-waves, indirect waves; L1-L5, cortical layers 1 to 5; PTN, pyramidal tract neuron. Open circles represent excitatory interneurons, filled circles indicate inhibitory interneurons; light-brown dotted lines represent cortico-cortical connections. Bottom right corner: Schematic representation of a D-wave followed by 4 I-waves. Inspired by a figure from Di Lazzaro & Rothwell, 2014.

However, TES and TMS induced currents may not stimulate neural components at the exact same locations. In TES, electric field runs from one electrode to another creating a rather more perpendicularly field to the cortex activating the PTN at an axonal level close to the cell body that leads to quite short-latency D-waves, therefore the peripheral response will also have a shorter latency. Soon after the description of the D- and I-waves due to TES application, several research groups (Burke et al., 1993; Day et al., 1989; Di Lazzaro, Oliviero, et al., 1998) described and compared similar epidural waves and their linked muscle responses after TMS over M1 in humans. The aforementioned complex group of evoked epidural waves had an overall slightly delayed latency when recorded after TMS pulses. Likewise, peripheral EMG responses to TMS at just supra-threshold intensities appear also about 1.5 ms later than TES-evoked responses. This difference in latency becomes smaller with increasing TMS intensities translating the elicitation of larger waves but also the recruitment of a more complex group of neural cells coming from a more extensive cortical area.

It is important to mention, though, that Burke et al. (Burke et al., 1993) registered the epidural waves from patients that were undergoing spinal surgery, and Di Lazzaro and co-workers (Di Lazzaro, Oliviero, et al., 1998) performed their experiments on patients that had cervical and thoracic epidural stimulating electrodes for chronic pain treatment. Due to the difficulty of exploring these processes in healthy humans and in order to better explain the complex current-brain interactions and their effect on cortical circuits, lately some international research groups have used complex computational models to investigate the generation of D- and I-waves within a magnetic field (Rusu, Murakami, Ziemann, & Triesch, 2014; Seo, Schaworonkow, Jun, & Triesch, 2016; Triesch, Zrenner, & Ziemann, 2015). These studies have contribute to the knowledge of how TMS interacts with the brain surface and adding more complexity to the theoretical canonical model of Di Lazzaro and Rothwell (Di Lazzaro & Rothwell, 2014).

Knowing that the TMS-induced current-brain interaction is intricate, one can anticipate that several factors may impact that relationship. In general terms, the effects of the current-brain are mainly influenced by (1) physical factors, being factors that depend on the TMS device and its physical parameters; and (2) physiological factors.

With regard to physical properties, the most prominent influencing parameters are TMS devices, coil geometry and orientation in relation to the scalp (see *Section 2.2* of the present chapter for further explanation), as well as TMS-pulse parameters such as pulse shape and duration, and the direction of the induced electric current in the brain (Di Lazzaro et al., 2001; Salvador, Silva, Basser, & Miranda, 2011; Sommer et al., 2013; Di Lazzaro & Rothwell, 2014) (*Section 2.3* of the present chapter deepens on the influence of shape and direction of TMS induced currents).

Relative to brain physiology, some of the major factors known to influence this TMS-brain interaction include individual differences in optimal current direction (Balslev, Braet, McAllister, & Miall, 2007) and pattern of cortical sulcation (Silva, Basser, & Miranda, 2008; Salvador et al., 2011), coil-cortex distance (Kozel et al., 2000; McConnell et al., 2001; Stokes et al., 2013), and state-dependent factors (Silvanto & Pascual-Leone, 2008; Ridding & Ziemann, 2010). Expanded physiological factors that are relevant for this thesis include age, age-related and metabolic diseases that potentially change brain excitability and reactivity (*Section 2.4* of the present chapter extents on this relevant physiological factors).

The parameters that influence TMS-brain interaction and that are the most representative and important for the present work, will be described and discussed throughout the following sections.

2.2 Devices and coils

A TMS machine consists on a main unit device and a stimulating coil. This makes the design of TMS devices quite simple and straight forward. The main TMS unit device is a single or a battery of large capacitors triggered by a thyristor switch. This allows to discharge high-voltage and high-current rapidly changing electric pulses. Most commercial TMS devices express pulse intensity as a percent of the maximal device output, being that output the amount of voltage passed through the stimulating coil. The maximal stimulator output (MSO), which reflects the maximum power or intensity of the device, as well as the length of the TMS pulse vary between devices and therefore the stimulating parameters for a specific protocol with a particular device have to be calculated and adjusted accordingly. As the technology advanced, devices also started including specific pulse-shape circuitry that allowed TMS machines to generate pulses with different waveforms and durations. Today many shapes and durations are available, but the most utilized ones are biphasic and monophasic waveforms. Furthermore, most of the commercial devices are only able to create pulses with either of these two waveforms. Biphasic and monophasic waveforms, their specific properties and influence on the TMS-brain interaction will be discussed below in *Section 2.3* of the current chapter. To illustrate the differences between market-available TMS devices, Kammer and colleagues (Kammer, Beck, Thielscher, Laubis-Herrmann, & Topka, 2001) studied the influence of two TMS machines [Dantec (MagVenture) and Magstim 200 stimulator (Magstim Co. Whitland, Dyfed, UK)] on the stimulation intensity threshold for the motor cortex. The authors obtained different results depending on the waveform and the device. Magstim monophasic pulses showed greater efficiency than biphasic pulses (i.e. lower resting motor thresholds (RMTs) with monophasic than with biphasic pulses) whereas MagVenture monophasic pulses were less efficient than biphasic (i.e. lower biphasic thresholds, regardless of current direction, than monophasic). From this study we know that depending on pulse waveform/current direction that were chosen, different devices may have different total

stimulation strengths. How to calculate the stimulation intensity and the precise protocol characteristics of the experiments of the present work will be described in *Methodology* (*Chapters 4 – 6*).

Other physical device features that affect TMS-brain interaction are coil geometry and coil orientation in relation with the scalp. Many different types of coil geometries are available nowadays. The most commonly used shapes are circular or round coils, and figure-of-eight coils.

In circular coils, current flows in one direction only, which is active at the edges of the coil with minimal activation at the center, and generates a spherical magnetic field. Circular coils typically have an outer diameter 90 mm and can stimulate large but shallow areas of the brain (***Figure 2.3.*** **A**). Coil orientation will depend in which side of the coil is in contact with the scalp. As an example, when stimulating the motor cortex, preferably stimulated using posterior-to-anterior (PA) current in the cortex, the coil should be center over the scalp vertex as shown in ***Figure 2.4***. Notice that when a circular coil is centered over vertex, one of the motor cortices will be stimulated with PA whereas in the other the current will be anterior-to-posterior (AP). The relationship between the current direction of a TMS pulse and the different cells of the motor cortex will be discussed in more depth in Chapter 2.3. Nevertheless, it is worth anticipating that those neural elements activated by PA or AP currents have distinctive excitability properties and latencies. Namely, PA currents are able to activate the motor neuron monosynaptically, whereas AP currents initiate a more intricate cascade of activations through cortico-cortical connections. This will lead to larger motor evoked potentials (MEPs) in the PA stimulated cortex. The presence of a MEP and its amplitude after the stimulation of the cortex with AP will depend on the intensity of the pulse. When exploring the contralateral cortex, the side of the coil in contact with the scalp is in most cases just reversed.

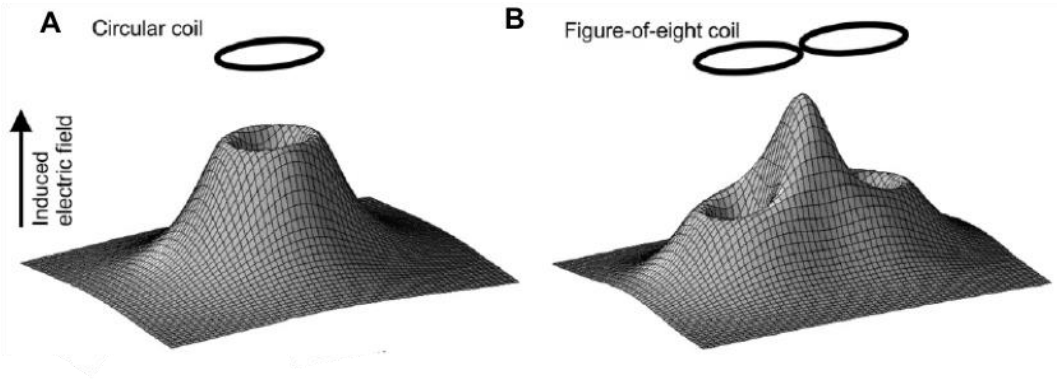

Figure 2.3. Distribution of the electric field induced by a circular coil (A) and a figure-of-eight coil (B). Modified from Hallett & Chokroverty, 2005.

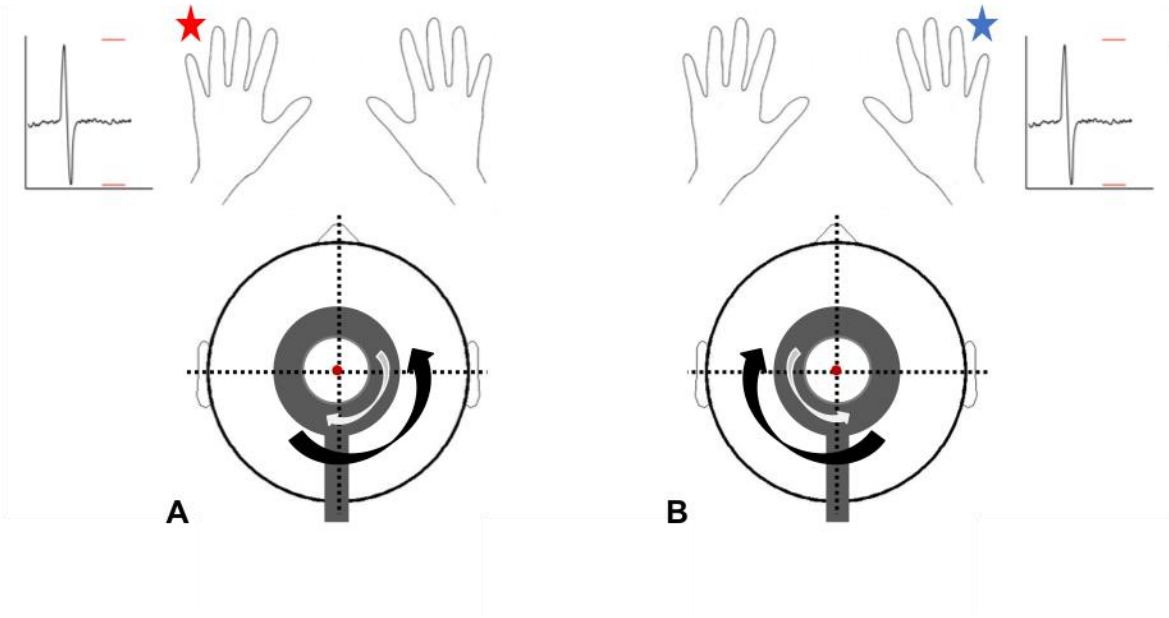

Figure 2.4. Axial representation of a head with a circular coil centered over the vertex or Cz.
The vertex of Cz is located in the intersection of the nasion-to-inion and ear-to-ear lines (red point), the light-grey arrows show the direction of the primary current in the coil, whereas the black arrows show the directionality of the TMS-induced current in the brain. In the case of ***Figure 2.4*** **A** the black arrow goes counterclockwise which means that the posterior-to-anterior (PA) component will fall over the right motor cortex eliciting a response in the left hand (red star). ***Figure 2.4*** **B** shows the black arrow in a clockwise direction; therefore, the PA direction is over the left motor cortex and responses in form of motor evoked potentials will be recorded from the right hand (blue star).

Nowadays the most commonly used coils both in clinical practice and research are figure-of-eight coils, probably due to their ability to focally activate a small portion of a targeted cortex. Thus, during the experiments of the present thesis, we chose to perform all the TMS protocols with this type of coil. A figure-of-eight coil combines two circular coils joined in the middle in a kind butterfly-shape conformation, for this reason these coils are also referred as butterfly-coils. In figure-of-eight coils current at each wing flows in opposite directions, resulting in summation of the currents at the junction of the wings where the summed magnetic field is more focal and stronger (***Figure 2.3*** **B**). The smaller the diameter of each wing of the coil the more focal the coil can be. On the contrary, the larger the diameter is, the deeper the magnetic field can reach. Most used coils have an outer diameter of 75 mm for each wing. The coil orientation depends on the current direction of the originated electrical field at the center of the coil (point of fields summation) and its relation to the scalp and the cortical target. The directionality of the combined current will, therefore, depend on the direction of each wing's current (see ***Figure 2.5***). In the motor cortex, several studies have investigated the influence of different scalp positions and their generated current directions in peripheral and spinal cord responses (Brasil-Neto, Cohen, Panizza, et al., 1992; Fuhr, Cohen, Roth, & Hallett, 1991; Mills, Boniface, & Schubert, 1992; Werhahn et al., 1994). From these studies we can resolve that the consensus for hand motor area is that the optimal coil orientation on the scalp is at an angle of 45º away from the midsagittal line (Brasil-Neto, Cohen, Panizza, et al., 1992) as shown in ***Figure 2.5*****.** However, coil orientation and position relative to scalp, pulse waveform and current direction will influence the outcome of TMS by activating particular neural components, as we will argue during the next section of this thesis.

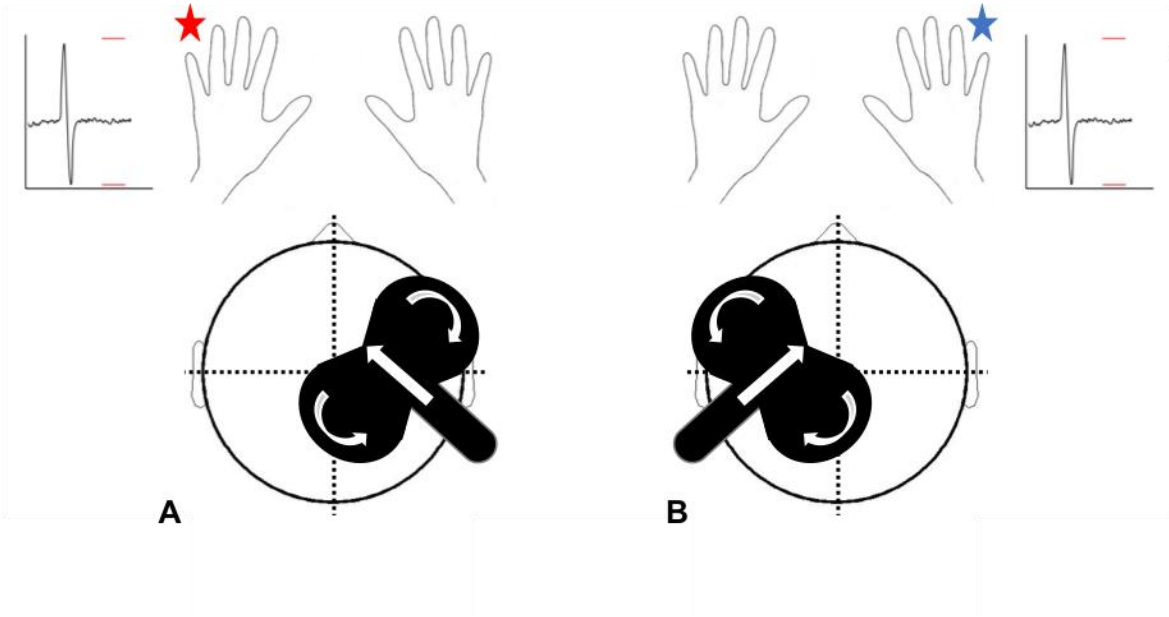

Figure 2.5. Axial representation of a head with a figure-of-eight coil over the right (**A**) or the left (**B**) hemisphere.
The coils are placed over the hand-representation area of the motor cortices in a 45º angle. The curved white arrows represent the TMS-induced currents being the straight white arrow the sum of both currents and the direction of the current in the brain. The straight white arrows are posterior-to-anterior in both cases (Figure **A** and **B**). When TMS coil is placed over right motor cortex, motor evoked potentials (MEPs) can be recorded on the left hand (red star). On the contrary, if the stimulated cortex is the left, MEPs will be elicited on the right hand (blue star).

2.3 Of waveforms and current directions. Modeling TMS-brain interactions

TMS waveform and current direction are parameters of great importance in order to understand the TMS-brain interaction and deepen into brain physiology. As we have already mentioned, specific waveforms and current directions activate particular neural components. Recently, many different pulse shapes or waveforms are available and a number of other waveforms have been explored for research purposes, but monophasic and biphasic

configurations are still the most common. These two types of waveforms are distinguished based on the length and duration of the first and second components of the induced current. Thus, biphasic pulses generate two equal phases in opposite directions, that is full positive/negative voltage oscillations resembling a cosine waveform. Hence, with a figure-of-eight coil at a preferential 45º angle over motor cortex, biphasic waveforms can either be AP-PA (bi_{AP-PA}) or PA-AP (bi_{PA-AP}). Whereas monophasic have a strong and sharp first rise in one direction and slow and long second component or decay towards baseline. Subsequently, monophasic waveforms can either be monophasic PA ($mono_{PA}$) or monophasic AP ($mono_{AP}$). ***Figure 2.6*** shows and schematic representation of monophasic and biphasic waveforms. While in monophasic shapes the first rise is the relevant part in terms of cortical stimulation, in biphasic both phases of the sine play an important role activating the cortex depending on stimulus intensity. At threshold intensities, the second phase of the biphasic shape is believed to have more impact; however, as we increase intensity, the first phase of the waveform becomes more relevant in terms of the cell populations that are activated (Di Lazzaro et al., 2011; Barker, 2017). Note that other scalp positions may lead to a different direction of the induced current and thus activate neural components differently. Since for the experiments of the present work, monophasic and biphasic were the shapes chosen with the coil at a preferential 45º angle over motor cortex, we will concentrate on the discussion of the influence of the latter configurations.

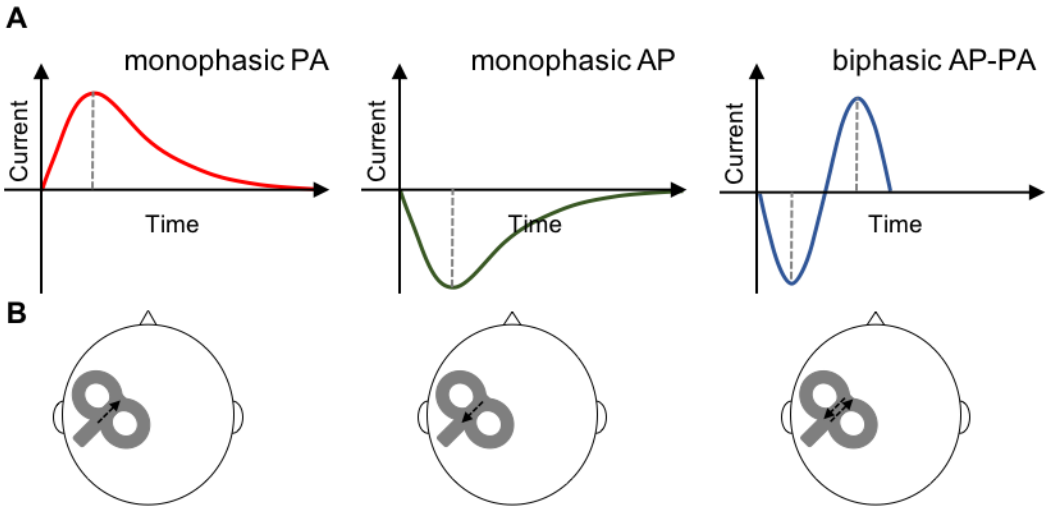

Figure 2.6. TMS pulse waveforms and induced current directions.
A. Diagram showing monophasic posterior-anterior (PA), monophasic anterior-posterior (AP), and biphasic AP-PA TMS pulse waveforms. **B.** Diagram showing location of the TMS coil over the left primary motor cortex with arrows depiction the direction of the induced current(s) in the brain.

Of particular relevance for the present thesis, some NIBS research groups have studied the influence of coil geometry, waveform and current direction on the motor outcome at a peripheral and spinal cord level (Amassian et al., 1987; Nakamura, Kitagawa, Kawaguchi, & Tsuji, 1996; Di Lazzaro et al., 2001; Di Lazzaro et al., 2001; Di Lazzaro et al., 2003; Di Lazzaro et al., 2004; Di Lazzaro et al., 2003; Di Lazzaro & Rothwell, 2014). During their experiments, the motor responses after either TES or TMS were recorded both in the muscle, in form of MEPs, and through cervical epidural electrodes. These cervical recordings made possible to quantify the impact of the above-mentioned TMS pulse parameters on D- and I-waves (the physiological bases of D- and I-waves are described in *Section 2.1* of the current chapter). As the results from epidural recordings demonstrated, depending on the intensity of the single-pulse TMS, different

waveforms and current directions show a selective recruitment of neural components. ***Figure 2.7*** represents a summary of their main findings.

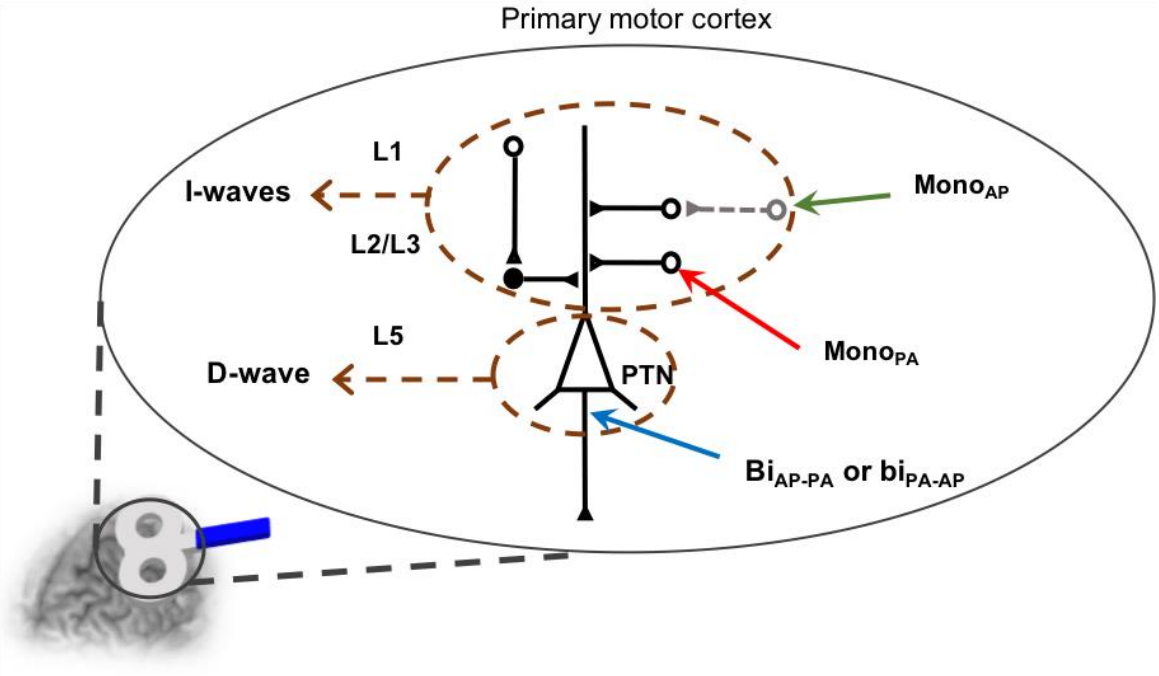

Figure 2.7. Schematic representation of motor cortex neural components and their interaction with TMS waveforms and current directions.

Abbreviations: Bi$_{AP-PA}$, biphasic anterior-posterior—posterior-anterior; Bi$_{PA-AP}$, biphasic posterior-anterior—anterior-posterior; mono$_{AP}$, monophasic anterior-posterior; mono$_{PA}$, monophasic posterior-anterior; L1-L5, cortical layers 1 to 5; PTN, pyramidal tract neuron. Open circles represent excitatory interneurons, filled circles indicate inhibitory interneurons; light-brown dotted lines represent cortico-cortical connections. Inspired by Di Lazzaro & Rothwell, 2014.

Monophasic waveforms tend to activate the PTNs indirectly. So, mono$_{PA}$ at low stimulus intensities elicits an I1-wave (early I-wave) whereas as the intensity increases, later I-waves begin to appear in addition to the I1-wave and with an approximate periodicity of 1.5 ms. In contrast, mono$_{AP}$ tend to first evoke late I-waves that have longer latencies and are more dispersed than the late I-waves elicited by mono$_{PA}$. In sum, the I1-wave has the lowest threshold with mono$_{PA}$

pulses, while on the contrary the late I-waves (I3-wave in particular) have the lowest threshold with $mono_{AP}$.

Biphasic waveforms have a more complex sinusoidal shape were both PA and AP directions are included in a specific order. Therefore, the pattern of elicited D- and I-waves becomes more heterogeneous accordingly. Given the complexity of biphasic pulses, stimulus intensity is of greater relevance. As previously mentioned, around threshold intensities, the last half of the sine wave seems to have larger influence on the stimulation of the motor cortex. With increasing intensities, the first phase of the wave starts activating neural elements and hence gaining relevance in the overall response. On this basis, biphasic pulses at high intensities tend to be less direction-dependent (Di Lazzaro et al., 2003; Barker, 2017) and is the intensity is sufficiently high, biphasic pulses are believed to directly activate the PTN. Nevertheless, the TMS-brain interaction with biphasic pulses is more intricate and the relationship between AP and PA components in a suprathreshold pulse is less known.

2.4 Transcranial Magnetic Stimulation in healthy and pathological aging

Once the main biophysical aspects of TMS and their influence in the TMS-brain interaction have been discussed, attention should be also paid to factors that are less controllable but of special relevance nevertheless. For the purposes of the present work, we will focus on healthy and pathological aging processes and their influence on the responses to TMS protocols.

Today, it is mostly assumed that brain changes across lifespan belong to dynamic, life-long and continuous developmental processes that imply both a loss in function but also neurocognitive benefits (Park & Reuter-Lorenz, 2009; Pascual-Leone & Taylor, 2011). When these changes during advancing age are subtle they fall into the context of normal aging. On the other hand, if they are severe they may lead to impairment of plasticity mechanisms, neuropathological processes, and eventually to certain medical conditions (e.g., AD). In this

context, defining plasticity and its mechanisms becomes essential. Plasticity mechanisms refer to the ability of the nervous system to adapt to internal and external changing conditions in the environment. These physiological mechanisms include both processes that are advantageous (e.g., learning and memory), as well as changes related to trauma or disease that represent a maladaptive plasticity (e.g., chronic pain, neural reorganization following stroke, etc.). This general definition of cortical plasticity includes both synaptic plasticity (i.e., Hebbian learning, long-term potentiation [LTP] and depression [LTD]), as well as non-synaptic plasticity processes (i.e., structural and functional changes in brain networks).

Related to the physiology of motor pathways with increasing age, neuroimaging studies (Ward & Frackowiak, 2003) have shown an association to cortical and subcortical activation of the motor system through growing complex compensatory central mechanisms. NIBS techniques may also be of great help providing additional information about changes in cortical excitability and plasticity across lifespan. There are various approaches to assess the mechanisms of plasticity using TMS. The most common approach to characterize cortical reactivity and excitability involves the application of blocks of single pulses. Motor thresholds or amplitude of MEPs are examples of these measurements. Further information about the usual protocols to perform RMT and MEP amplitude will be described in *Methodology* (*Chapters 4 – 6*). Among the NIBS techniques, of special importance to evaluate brain's plasticity is rTMS and in particular the theta-burst stimulation (TBS) paradigm. A more comprehensive definition of TBS and its physiological implications will be explained in *Chapter 4 – Section 4.4*. TBS protocols have been able to demonstrate plasticity mechanisms and cortical excitability changes across lifespan showing a progressive and linear reduction in the responses to TBS with age (Freitas et al., 2011). Moreover, TBS has revealed altered neuroplastic mechanisms in autism spectrum disorders (Oberman et al., 2012), traumatic brain injury (Tremblay, Vernet, Bashir, Pascual-Leone, & Theoret, 2015), schizophrenia (McClintock, Freitas, Oberman, Lisanby, & Pascual-Leone, 2011), T2DM (Fried et al., 2017), and AD (Koch et al., 2012).

2.5 Reliability of Transcranial Magnetic Stimulation

Previous sections of this chapter have characterized the factors that may introduce variability in the TMS-brain interaction. As TMS is a quite powerful neurophysiological technique that has been increasingly adopted in both the scientific field and the daily clinical practice, it is very relevant to identify possible elements that contribute to unstable responses to TMS through time.

Fortunately, the effects of TMS on motor cortex are easily quantifiable. When applied over the primary motor cortex at sufficient intensity, TMS can activate the corticospinal pathway and elicit a muscle response. The MEPs represent the activation of a muscle after a single pulse and can be easily recorded and measured. Further information about the MEP physiology is provided in *Chapter 4 – Section 4.2*.

Given the increasing relevance of TMS and the existence of a measure that can be easily quantified, the study of the consistency, reliability and variability of TMS responses in form of MEPs becomes essential to interpret the experimental and clinical data available today as the variability of TMS responses could reduce its sensitivity to detect meaningful changes over time or to an intervention. These types of studies are the best source of statistical knowledge that allow us to assess the validity of TMS as a technique and evaluate its ability to discriminate between subjects or to differentiate between groups.

Numerous statistical methods are available to investigate the repeatability and reproducibility of a technique. To better understand how TMS works and how stable its responses are, we will focus on intra-subject reliability studies. These studies evaluate the consistency of the data obtained in a group of subjects when the TMS is performed either by one or several raters or in one or more visits. The most suitable statistical method is the intra-class correlation coefficient (ICC). This coefficient was first suggested by McGraw and Wong (McGraw & Wong, 1996) and quantifies how much of the observed variability of a response belongs to the heterogeneity of the

sample and how much to visit-to-visit or inter-rater variability. Therefore, ICC values of 1 reflect the maximum reliability of a measure, and 0 indicates no reliability. When sample sizes are small, ICC values can become negative if the within-group variance exceeds the between-groups variance (Kenny, Mannetti, Pierro, Livi, & Kashy, 2002). There is no consensus in the literature about a classification of ICC values into categories. Nonetheless, in the present thesis we decided to use the most common range in categorizing reproducibility in neurophysiological assessments (Portney & Watkins, 2009) as well as in TMS literature:

ICC values:

> 0.75 *high* reliability

0.5 - 0.75 *moderate* reliability

0.25 - 0.5 *low* reliability

< 0.25 *very low / no* reliability

According to this range, an excellent reliability translates that less than 25% of the variance of the sample comes from random measurement errors (e.g. comparison of different visits, or between different raters) and more than 75% of agreement.

For the purposes of this thesis and in order to understand the state of the TMS field, a brief literature review was conducted in relation to TMS and its reliability and/or reproducibility emphasizing studies investigating ICC on the most relevant single- and paired-pulse protocols for the intrinsic hand muscles, including RMT, MEP latency and amplitude, cortical silent period (cSP)

and inhibitory and facilitatory paired-pulse protocols (all of the above are described in depth in *Methodology* (*Chapters 4 – 6*)). We found relatively few publications that full filled these criteria (Bastani & Jaberzadeh, 2012; Carroll, Riek, & Carson, 2001; Christie, Fling, Crews, Mulwitz, & Kamen, 2007; Fleming, Sorinola, Newham, Roberts-Lewis, & Bergmann, 2012; Hermsen et al., 2016; Hoonhorst, Kollen, Berg, Emmelot, & Kwakkel, 2014; Kamen, 2004; Kimiskidis et al., 2004; Liu & Au-Yeung, 2014; Livingston & Ingersoll, 2008; McDonnell, Ridding, & Miles, 2004; Ngomo, Leonard, Moffet, & Mercier, 2012; Sankarasubramanian et al., 2015; Schambra et al., 2015). Among the found publications, it is important to point out a systematic review of the literature carried out by Beaulieu and colleagues (Beaulieu, Flamand, Masse-Alarie, & Schneider, 2017). This review addressed the most important methodological issues of different studies in different muscle groups. Unfortunately, the authors did not further investigate the results of the articles included. Nevertheless, the review concluded that there is very limited evidence of TMS reliability, highlighting the need for a greater number of studies on this particular theme. In addition, they provided recommendations for future studies not to be affected by the methodological and statistical problems that the authors observe in the data published to date.

With the purpose of illustrating in a clear manner the evidence up to now, ***Figure 2.8*** shows all the data found after our brief review on reliability coefficients, ICCs.

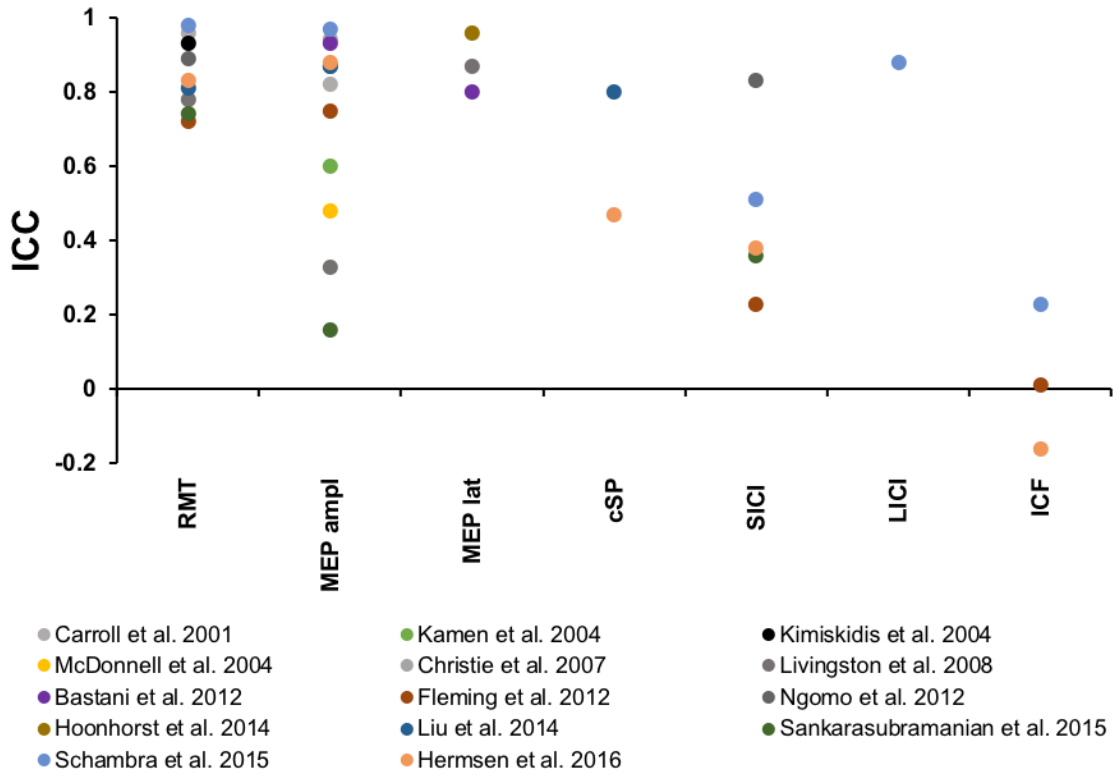

Figure 2.8. The graph shows the results of intra-class correlation coefficients (ICCs) of the studies found in the brief literature review.
These studies evaluated the reliability of single- and paired-pulse TMS protocols over the intrinsic hand muscles. *Abbreviations*: cSP, cortical silent period; ICF, intracortical facilitation; MEP ampl., amplitude of the motor evoked potentials; MEP lat., latency of the motor evoked potentials; LICI, long-interval intracortical inhibition; RMT, resting motor threshold; SICI, short-interval intracortical inhibition.

Single-pulse protocols shown in ***Figure 2.8*** include RMT, MEP latency and amplitude, and cSP. For paired-pulse paradigms we decided to cover the ones that are more frequently described in literature, that means short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI) and intracortical facilitation (ICF).

The intensities of most of TMS protocols are based on RMT values, consequently RMT is the variable which is the most studied in literature. As shown in ***Figure 2.8*** reflects RMT is also

the most stable and reliable measure of TMS together with MEP latency. However, the evidence for cSP and paired-pulse protocols is very scarce and scattered, hence the community will need many more studies before being able to reach any conclusions about their consistency and reliability. Another important feature to point out is that during the studies of the reviewed articles, TMS was mostly performed only with monophasic waveform and PA current direction in the cortex. It is noteworthy as well, the broad spread of the data for MEP amplitude. This huge variability is of a great relevance given that most of the studies and publications report the mean amplitude of a number of MEPs as their baseline.

The dispersion of reliability data most probably occurs as a consequence of a great heterogeneity in the application of TMS across the different studies included in this brief review. Even though the diversity of ICC results has multiple sources that are not fully understood nowadays, attention should be drawn to several factors that have considerable influence in the responses. Overall the factors that may be influencing TMS responses could be divided into extrinsic (or technical parameters of TMS) and intrinsic (characteristics of the subject).

As a reminder, different TMS devices or gadgets have particular maximal output strength or pulse characteristics such as pulse width, that have been found to change TMS responses (D'Ostilio et al., 2016; Hannah & Rothwell, 2017; Lang et al., 2006). In order to account for some of those particularities and calibrate the pulse strength, most researchers have related the stimulation intensity of the TMS pulses to a % of the RMT, which is itself a % of MSO. Other pulse characteristics including waveform and current direction (previously discussed in detail in *Section 2.3* of the present chapter) are important sources of variability when trying to elucidate reliability among different studies. Moreover, different types of coil geometry will influence the characteristics of the pulse and the interaction of the electric field with the cortex (see *Section 2.2* of the present chapter). The use of neuronavigation can provide with a stability of a chosen location preventing from minor movements around the stimulation spot and consequently less variability on MEP responses (Julkunen et al., 2009). Recently, two studies (Chang et al., 2016;

Goldsworthy, Hordacre, & Ridding, 2016) have highlighted the importance of the total number of pulses when averaging MEP amplitude or latency. Both studies reached to the conclusion that in order to get moderate to high reliabilities it is essential to average at least 20 to 30 responses with the same pulse characteristics for MEP amplitude. MEP latency showed much lesser variability in both studies.

Physiological or intrinsic factors, in turn, can be classified into non-modifiable (e.g. age, gender or genetics) and modifiable (e.g. brain state, cortical activity or metaplasticity).

Age and gender have been probed to influence the response to specific TMS protocols (Pitcher, Ogston, & Miles, 2003; De Gennaro et al., 2003). In particular, age related physiological changes such as reduction in the total number of activated motor neurons or a loss of synchronous activation may be playing a relevant role in the variability of responses to TMS. However, very few studies have investigated the influence of age in TMS reliability including subjects over the age of 50 (Fleming et al., 2012; Kimiskidis et al., 2004) and only one study exclusively recruited subjects over 65 years (Christie et al., 2007).

During the last years, genetic polymorphisms have emerged as relevant intrinsic factors that might be associated to particular patterns of response after TMS protocols. Regarding the present work, Brain-derived neurotrophic factor (BDNF) and its polymorphisms are worth noting. BDNF is a growth factor that is greatly expressed in the nervous system, crucial in brain development and involved in LTP and LTD (Gottmann, Mittmann, & Lessmann, 2009). The BDNF polymorphism (BDNF Val66Met) in humans has been shown to influence learning and memory processes and reduce cortical plasticity as well as modify responses to single-pulse TMS (Cirillo, Hughes, Ridding, Thomas, & Semmler, 2012). Val66Met carries also show reduced responses to repetitive protocols of TMS (Cheeran et al., 2008).

Studies in patient populations showed that pathologies such as stroke may change the reliability of TMS (Cacchio et al., 2011; Schambra et al., 2015) and that the variability of the response depends on whether the tested hemisphere is the affected or not.

In addition, modifiable factors such as the level of drowsiness or attention (Mirdamadi, Suzuki, & Meehan, 2017) and different brain states (Silvanto & Pascual-Leone, 2008) change cortical excitability and therefore the response to TMS. Hence, TMS responses may change based on the brain state or in other words, the impact of TMS depends not only on the characteristics of the pulse but also on the previous cortical activity and the sensitivity of the neural networks to the stimulus at a given time. As a practical example, muscle voluntary activation right before TBS (see *Methodology* (*Chapters 4 – 6*) for a comprehensive description of TBS) change the outcome of this rTMS protocol (Huang, Rothwell, Edwards, & Chen, 2008). Metaplastic processes, or the history of synaptic plasticity, also influences the effects of TMS as demonstrated by Gamboa et al. (Gamboa, Antal, Moliadze, & Paulus, 2010), who obtained paradigmatic results after lengthening ordinary TBS protocols. Also related to brain metaplasticity, Siebner and co-workers (Siebner et al., 2004) applied tDCS prior to low-frequency rTMS changing the outcome of the rTMS paradigm probing how homeostatic plasticity (namely intrinsic neural mechanisms that regulate the network excitability after a given stimulus) affects TMS-brain interactions.

Although, we know some of the factors that influence the responses to rTMS protocols, very few studies have investigated the reliability of different repetitive protocols. Maeda and colleagues (Maeda, Gangitano, Thall, & Pascual-Leone, 2002) studied high- (20Hz and 10Hz) and low-frequency (1Hz) rTMS finding that 20Hz had fair reliability but 1 and 10Hz reliability's was poor. The groups of Hinder, Schilberg, Vernet and Vallence studied TBS using its common presentations, both intermittent (iTBS) (Hinder et al., 2014; Schilberg, Schuhmann, & Sack, 2017) and continuous (cTBS) (Vallence et al., 2015; Vernet et al., 2013) in young healthy controls (age under 44 years), showing fair to poor reliability in both cases. Previously identified factors that introduce variability to post-TBS measures are prior exercise (McDonnell, Buckley, Opie, Ridding, & Semmler, 2013), ongoing voluntary activity (Iezzi et al., 2008), and other state-dependent effects (Silvanto & Pascual-Leone, 2008), that can influence the efficacy of TBS and thus increase intra-individual variability (for a review, see Ridding & Ziemann, 2010).

A series of studies included in this thesis aim to look into some of the physical and physiological factors that may affect TMS reliability in scenarios in which attention has not been carefully and sufficiently paid. Firstly, we focused on TMS parameters such as waveforms and current direction on young healthy volunteers in order to elucidate their influence on single- and paired-pulse TMS protocols. Secondarily, and given the limited data on physiological factors such as age (i.e. healthy older healthy controls) and age-related diseases (i.e. T2DM and AD), we conducted a TMS study on their influence on a single-, paired- and repetitive TMS (i.e. TBS). Finally, the cohorts of younger and older healthy controls were compared for specific age-related impact on single- and paired-pulse TMS outcomes.

2.6 Safety of Transcranial Magnetic Stimulation

When dealing with a medical device the safety of the procedures must be thoroughly investigated. Two consensus conferences have been held to establish a safe use and recommendations for TMS both in academic and clinical environments. The publication that came out after those conferences still remain the safety guidelines for TMS (Rossi, Hallett, Rossini, Pascual-Leone, & Safety of TMS Consensus Group, 2009). Moreover, this safety guidelines proposed by The Safety of TMS Consensus Group were the working basis for the TMS safety forms that we used to screen possible contraindications and side effects during the experiments of the present thesis.

As described previously in the safety guidelines, TMS is a very safe non-invasive way of exploring the brain. ***Table 2.1*** presents the reported side effects. Seizures are the most severe side effect and a major safety concern but they are also extremely rare. Theoretically, seizures can occur during two time windows, first, during the time of stimulation, and second, post-stimulation due to the modulation of cortical excitability (i.e. kindling effect). In case of a seizure, TMS should be ceased and standard seizure treatment should be started. An important differential

diagnosis, and probably an underestimated TMS side effect, is syncope or fainting spells, over all if the presentation is in form of convulsive-syncope.

Table 2.1. Potential side effects of TMS. Modified from Rossi et al., 2009.

Side effect	SP TMS	PP TMS	LF rTMS	HF rTMS	TBS
Seizure induction	+	NR	+	++	++
Syncope	Possible epiphenomenon non-related to direct brain effect				++
Headache, local pain, neck pain, toothache	++	++	+++	+++	++
Transient hearing changes	++	NR	++	++	NR
Transient acute hypomania induction	No	No	+	+	NR
Transient cognitive/ neuropsychological changes	NR	NR	+	+	+
Burns from scalp electrodes	NR	NR	NR	+	NR
Induced currents in electrical circuits	Only if close proximity of TMS with an electrical device				
Structural brain changes	NR		Inconsistent		NR
Histotoxicity	NR		Inconsistent		NR
Other biological transient effects	NR		NR	TSH, and blood lactate level	NR

Abbreviations: NR not reported; + rare; ++ possible; +++ frequent. SP, single-pulse; PP, paired-pulse; TMS, transcranial magnetic stimulation; LF, low frequency; HF, high frequency; rTMS, repetitive transcranial magnetic stimulation; TBS, theta burst stimulation; TSH, transient hormone.

Acute mania and psychiatric changes in mood have been reported after rTMS protocols for uni- and bipolar depression. Thus, it is important to screen and monitor mood changes as a safety routine for TMS exposure.

Local pain, headache and local discomfort are the most commonly reported side effects (up to an estimated 20-40% of the subjects that undergo TMS (Rossi et al., 2009)). Pain and discomfort usually do not last much after a TMS session and common analgesic treatment is normally enough for headaches that may overlast the time of stimulation. The intensity of the pain related side effects depends on individual susceptibility and specific characteristics of TMS parameters, such as intensity and frequency, coil type or scalp location.

Another important common side effect is acoustic trauma due to loud TMS clicking noise that can be of the order of 120-140 dBs. This side effect can be easily avoided using earplugs as hear protection for both the subject or patient and the technician that applies TMS.

A second important point of the safety guidelines are contraindications of TMS. Following the safety guidelines, the only absolute contraindication is intracranial electrodes when metallic hardware is in contact with the TMS coil discharge. The guidelines recommend to avoid using TMS or carefully monitor the sessions in people with implanted cranial electrodes (no direct contact) or cochlear implants; history of syncope, seizure or epilepsy; cerebral lesions; drug intake that may interact with TMS or recent drug withdrawal, and pregnant women or pediatric populations.

Since the disclosure of the safety guidelines in 2009, several studies and reviews have been published in relation to some of the previously mentioned contraindications. Deng and colleagues (Deng, Lisanby, & Peterchev, 2010) have proposed specific parameters for TMS on patients treated with DBS. More recently a Cochrane review has been published (Chen, Spencer, Weston, & Nolan, 2016) on TMS as a safe and effective treatment for epilepsy. Several authors have studied TMS as a treatment tool for certain cerebral lesions (such as stroke) or drug abuse. A recent review on how TMS might affect the brain of children and adolescents has been

presented (Hameed et al., 2017). Even though, the authors claim that there is evidence of clear and safe therapeutic and neurophysiological potential, more research is still needed considering developmental specificities.

3 Transcranial Static Magnetic Stimulation

3.1 Fundamentals and mechanisms of action

Magnetic fields can be classified into dynamic magnetic fields (DMF) and static magnetic fields (SMF), depending on whether there is a change of the direction or the intensity of the field over time that is associated with an induced electric current in accordance with Faraday's law of induction. An example of a NIBS technique that uses time-varying magnetic or DMFs to modify brain physiology is TMS, that has been discussed in the previous chapter.

In relation to magnetic fields that do not change over time, many studies have evidenced that SMFs have a great influence on biological systems. For instance, many animals rely on Earth's magnetic field for spatial orientation and navigation. So as to study the SMF and their impact on organisms, the scientific community has suggested a classification where weak fields are those less than 1mT, moderate are 1mT to 1T, strong include 1 to 5T, and ultrastrong fields are those greater than 5T (Rosen, 2003).

A number of studies have shown that many organisms, including vertebrates, respond and have the ability to use geomagnetic fields, which are weak magnetic fields, in order to orient themselves in space even in complete darkness as well as having a preferential position towards the magnetic north (Holland, Thorup, Vonhof, Cochran, & Wikelski, 2006; Lohmann, 1993; Tian, Pan, Metzner, Zhang, & Zhang, 2015).

Likewise, strong and ultrastrong fields can alter the preferred orientation of a variety of diamagnetic anisotropic organic molecules (Rosen, 2003), they change monkeys' EEG while exposed to fields from 2 to 9T (Beischer & Knepton Jr, 1966) or modify their visual behavior when exposed to even stronger fields (4.6-7 T) (Thach, 1968). A widely-known example of a commonly used device in clinical practice of strong and ultrastrong fields is MRI equipment. Nonetheless, the evidence of the influence of those fields in the human nervous system is less clear. Since the clinical use of higher electromagnetic field MRIs has been extended, a number of publications have pointed towards transient but significant modifications on cortical excitability at a motor, sensory or cognitive levels as well as peripheral activation in form of tingling sensations or pain (Arrubla, Neuner, Hahn, Boers, & Shah, 2013; Schlamann et al., 2010; Shellock & Crues, 2004). While others have evidenced an increasing number of reported transitory adverse events such as vertigo, nausea, phosphenes or metallic taste (Kim & Kim, 2017; Theysohn et al., 2008).

Moderate SMFs have also been utilized to transiently modify brain 's reactivity and excitability in a controlled manner. The effects of the moderate SMFs are not yet utterly understood, but at a cellular level could be explained by the diamagnetic and anisotropic characteristics of the phospholipids that alter the ion channels and the calcium ion flux of the membrane (Rosen, 2003), the effects of ferromagnetic particles that are present in the brain over ion channels (Dobson & St Pierre, 1996), or to the Hall effect on voltage-dependent channels (Balcavage et al., 1996), albeit more controversial (St Pierre & Dobson, 2000). Furthermore, different animal experiments have shown a variety of electroencephalographic and behavior effects that overcome the time of exposure to moderate SMFs. Coots and colleagues have demonstrated inhibition in spinal cord conduction of guinea pigs (Coots, Shi, & Rosen, 2004), another study (Aguila, Cudeiro, & Rivadulla, 2016) found that direct exposure to SMFs of up to 75 min transiently impaired detection of visual stimuli and increased the reaction time in monkeys and decreased visual cortex excitability in anesthetized cats when neural spiking activity was directly recorded. Finally, some other groups have gone further and investigated the use of the SMFs for pathological conditions

such as epilepsy in animal models (McLean, Engstrom, Holcomb, & Sanchez, 2003; McLean, Engstrom, Qinkun, Spankovich, & Polley, 2008). These studies suggest that SMFs may induce temporal plastic changes, specifically, LTD-like plastic changes. In the context of the application of moderate SMFs to the nervous system, a novel technique has been recently described for human use. The so called tSMS, is a NIBS technique that, unlike TMS that requires a rapid change in an electrical field, utilizes moderate SMFs that are not associated with electrical fields.

3.2 Previous studies and background

In 2011 Oliviero and co-workers (Oliviero et al., 2011) demonstrated that the application of moderate (45 MGOe; megagauss-oersteds nominal strength 628 N (64 kg) ≈ 0.5 tesla-T) SMFs on the primary motor cortex was able to produce a significant attenuation of cortical excitability. During their experiments, the authors applied a Neodymium magnet over M1 for 10 minutes and the TMS-induced MEPs showed an average decrease of around 25% of peak-to-peak amplitude. The reduction lasted for at least 6 minutes beyond the application with posterior complete recovery of the MEP amplitudes (***Figure 3.1*** shows the positioning of the magnets as well as the main results of the experiments). They also carried out different experiments where they tested whether the polarity (north and south), the size of the magnet (the bigger magnet was 45 mm diameter, the smaller was 30 mm diameter), or the time of exposure (exposures of 1, 5 and 10 min) may influence the effects of SMFs. They conclude that the bigger magnet and at least 10 min of exposure were required in order to decrease motor cortex excitability, whereas the polarity did not play an important role. Finally, the authors also tested the responses measured with TES instead of TMS. As briefly mentioned in the introduction of *State of the Art* (*Chapters 2* and *3*), TES bypasses the cortex and directly activates the subcortical fibers. Therefore, TES has been an extensively used NIBS technique to differentiate whether the effects of a given protocol belong to cortical or subcortical activation of the corticospinal tract. The authors did not find any changes

in stimulation thresholds neither in MEP amplitude when the motor system was evaluated with TES, thus confirming the cortical origin of the inhibitory processes after tSMS.

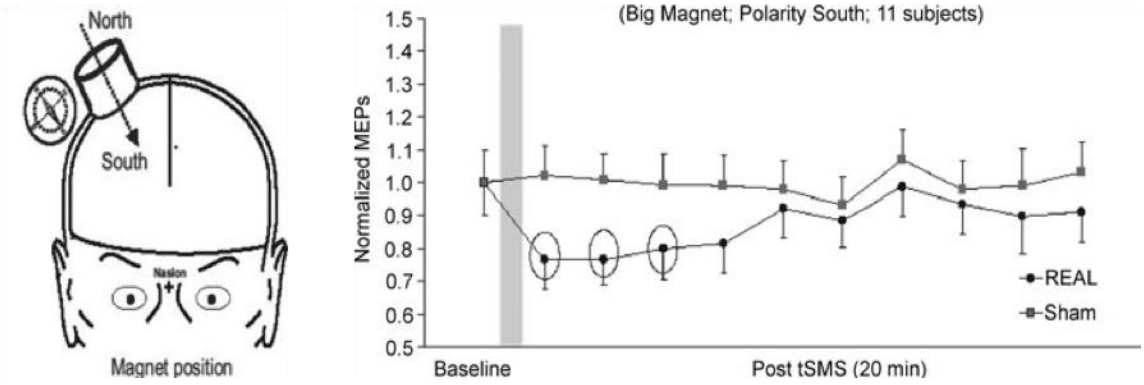

Figure 3.1. Results on MEP amplitude after the application 10 minutes of transcranial static magnetic stimulation (tSMS) on the motor cortex.
The head representation on the left shows a depiction of the magnet positioning over motor cortex during the intervention. The main results after 10 minutes of real or sham interventions are presented on the graph on the right. The light grey column symbolizes the time of intervention and the circled time points show the significant inhibition of the MEP amplitude after real tSMS. Modified from Oliviero et al., 2011.

Few years later, a first independent group (Silbert, Pevcic, Patterson, Windnagel, & Thickbroom, 2013) replicated the reduction in motor cortex excitability. They also showed an increase of RMT which was related to the decrease in MEP amplitude. Moreover, Nojima and colleagues (Nojima, Koganemaru, Fukuyama, & Mima, 2015) further investigated intracortical inhibitory processes of the motor cortex. They found that TMS-elicited γ-aminobutyric acid $(GABA)_A$-dependent inhibition (i.e. SICI; see *Chapter 4 – Section 4.3* for physiological connotations) was enhanced after 15 min of tSMS, suggesting that GABA-related interneurons were responsible for the depression of cortical excitability. This GABA-dependent effect, though, is possibly related to specific cortical neural networks as shown by Arias and coworkers (Arias,

Adan-Arcay, Puerta-Catoira, Madrid, & Cudeiro, 2017). The authors did not find increased inhibition after two TMS protocols that explore GABA-inhibitory interneuron networks different from those evaluated by SICI (i.e. Short and long afferent inhibition, SAI and LAI, respectively). In addition to these findings and supporting the idea of an specific local cortical effect, Matsugi and Okada (Matsugi & Okada, 2017) applied tSMS to the right hemisphere of the cerebellum and evaluated both the M1 excitability and cerebello-brain inhibition (CBI). Their results showed a local inhibitory enhancement of CBI with no effects on M1 MEP amplitude.

Finally, it has been shown that the influence of tSMS is not only limited to motor cortex. Several groups have investigated the effects of SMF over visual (Gonzalez-Rosa et al., 2015) and parietal (Carrasco-Lopez et al., 2017; Kirimoto, Asao, Tamaki, & Onishi, 2016; Kirimoto et al., 2014) cortices with similar inhibitory findings. The application of tSMS increased EEG power in the alpha range in both the visual and the parietal cortices. This increase in alpha power was also related to an impairment of visual and parietal tasks performance and a reduction of the amplitude of somatosensory evoked potentials, specifically the N20 when the tSMS was over the somatosensory cortex and the N30 when it was over M1.

In summary, the easy portability due to its low weight and the low cost of this NIBS technique as well as all the findings described above, lead to think that in the near future the clinical use might be possible for those pathological conditions where there is an abnormal increase of cortical excitability. Aligned with this, two studies have started examining the potential use of tSMS in photophobia during migraine (Lozano-Soto et al., 2017) and motor impairment in Parkinson Disease (Dileone et al., 2017) with promising results.

3.3 Safety of Transcranial Static Magnetic Stimulation

In a publication in 2005, Miyakoshi (Miyakoshi, 2005) reviewed the possible effects of the SMFs at a cellular level. The author summarized the results of several studies from different research groups conducted on various cells from different parts of the body reaching to the conclusion that SMFs do not remarkably affect cell growth or toxicity even though they might affect c-Jun gene expression as an anti-apoptotic factor. More importantly, Miyakoshi mentioned that the magnetic flux may have effects on the control of intracellular ions (particularly Ca^{2+}) but its effects most probably depend on the cell type.

Soon after the first studies on the application of SMFs on humans, three publications have explored both the magnetic fields and their decay with distance, and the safety of tSMS. In the first studies (Rivadulla, Foffani, & Oliviero, 2014; Tharayil, Goetz, Bernabei, & Peterchev, 2017), the authors modelled the magnetic flux and its effects calculating the amount of magnetic field that may reach the cortex (see *Chapter 5* for further information). They did their experiments on different magnet sizes and configurations in both experimental conditions (Rivadulla et al., 2014) and a theoretical human head model (Tharayil et al., 2017). In the third publication (Oliviero et al., 2015), Oliviero and colleagues evaluated the effects of SMFs in terms of cognitive/motor performance tests and biomarkers of brain cellular damage after a long exposure to the magnetic fields. They studied the safeness of a two-hour exposure to static magnetic fields in 17 young healthy participants. They measured the serum concentration of neuron-specific enolase (NSE) and protein S-100, sensitive markers of neural damage and glial activation respectively. They also tested several cognitive and motor performance tasks such as, Mini-Mental status examination (MMSE), Verbal fluency test, Nine-hole peg test (NHPT) and two-Choice Reaction time test. Neither changes in cognitive and motor tasks nor changes in NSE serum concentration were found. On the other hand, tSMS led to a post-exposure reduction of the S-100 that recovered after 24h. S-100 is believe to translate glial reactivity and damage, hence in any case tSMS would

yield glial protective effects. In consequence, they claimed that tSMS does not produce tissue damage or cognitive/motor performance worsening.

In addition to this last safety report, neither of the human studies already mentioned in *Section 3.2* of the present chapter, reported any side effects. Henceforth, considering all the data available to this date, tSMS seems to be a safe and reversible way of changing brain's excitability. Anyway, further studies will be needed to confirm the safeness of this novel NIBS technique and to explore further applicability for human use.

GENERAL METHODOLOGY: THEORETICAL FOUNDATIONS AND INSTRUMENTATION

The experiments of the present thesis were conducted on human adult participants. All the studies were carried out in accordance with the Declaration of Helsinki and were approved by the local Institutional Review Board. All participants provided written informed consent prior to enrollment and received monetary compensation upon completion.

In the present chapter, we will describe different methods, techniques, and protocols that were utilized in this thesis and related experiments. Furthermore, we will expand on their fundamentals and physiological implications.

All parameters used in the study met technical recommended standards by IFCN (Deuschl & Eisen, 1999; Nuwer et al., 1994) and were in agreement to current recommended guidelines for the safe application of TMS endorsed by the International Federation of Clinical Neurophysiology (IFCN) (Rossi et al., 2009; Rossini et al., 2015). In the case of tSMS where no published guidelines are available yet, the stimulation was performed following the parameters shown as safe in previous literature.

The specific parameters used in the studies of this thesis as well as the appropriate demographics of the samples will be described in the methods section of each particular experiment.

4 Transcranial Magnetic Stimulation

As mentioned in the *Introduction* (*Chapter 1*) of the present manuscript, TMS is a powerful technique that allows the study but also the modification of brain dynamics of specific areas of the cortex, their neural pathways and brain networks that are functionally and structurally connected to those areas. Single- and paired-pulse TMS can be used as a neurophysiological tool to evaluate intra- or inter-cortical excitability, integrity of different pathways (e.g. corticospinal motor tract) as well as evaluate pathophysiology of several diseases or its progression over time. Furthermore, TMS pulses could anticipate therapeutic results or monitor the efficacy of an intervention. The motor response of a target muscle after the activation of the corticospinal tract by TMS can be recorded by placing EMG electrodes. EMG general description and implication will be described in *Chapter 6 – Section 6.1*.

In addition, if TMS is applied in form of trains of pulses with an internal frequency, rTMS can induce plastic changes that overlast the time of exposure.

In the following section, we will describe the techniques we performed during the experiments of the present thesis using single- and paired-pulses and their physiological implications. Additionally, we will comment on TBS, a specific form of rTMS that was used in one of the experiments.

4.1 Motor hotspot and thresholds

4.1.1 Motor hotspot

When TMS is applied over motor cortex, the *hotspot* is the scalp position from where the largest and most consistent responses are elicited for a given target muscle. Therefore, the motor

hotspot becomes a perfect target for evaluating the corticospinal motor pathway. The identification of the motor hotspot can be improved using individual MRI-guided neuronavigation (Julkunen et al., 2009).

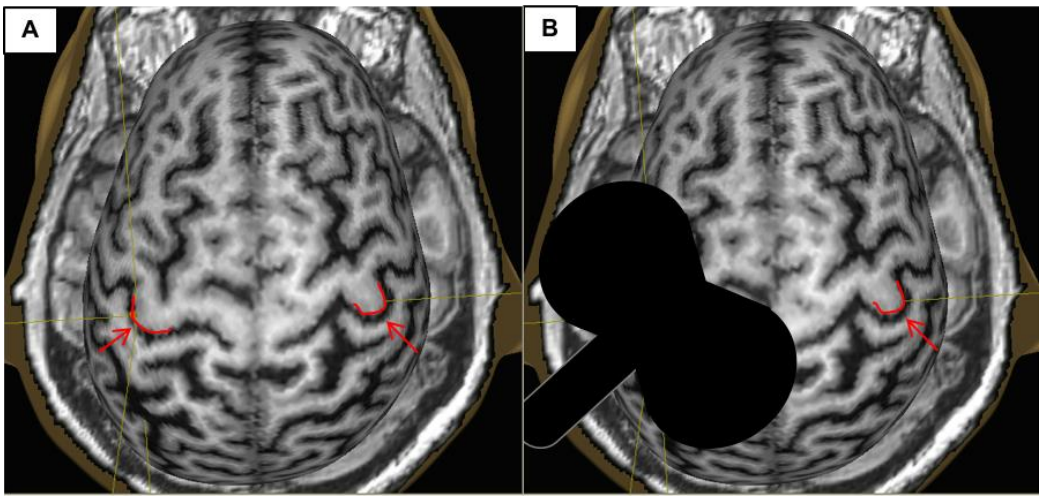

Figure 4.1. Hotspot search starting location with neuronavigation.
Figure 4.1A represents the axial view of the magnetic resonance imaging (MRI) of a reconstructed brain. Red lines and arrows mark the bilateral hand knob. **Figure 4.1B** shows the coil position in relation to the scalp and the landmarks for the hand cortical representation on the left hemisphere.

If neuronavigation is available, the search starts at the *hand knob* of the post-central gyrus for the hand muscles (see ***Figure 4.1A***). Neuronavigation helps to direct the TMS coil to specific places in neuronavigation. MRI scans can be used with neuronavigation systems to both identify a brain region and to consistently target that region within and across TMS sessions (Herwig et al., 2001; Ruohonen & Karhu, 2010). Furthermore, several studies have investigated the anatomical location and definition of the hot spot using neuronavigated TMS (nTMS) (Kantelhardt et al., 2010; Niskanen et al., 2010). As argued in *Chapter 2 – Section 2.4*, using MR-guided neuronavigation may reduce improve the reliability of TMS reducing individual variations and

eliciting more stable MEPs with greater amplitudes (Ahdab, Ayache, Brugieres, Farhat, & Lefaucheur, 2016; Danner et al., 2008; Julkunen et al., 2009). MRI navigated-TMS has been used throughout all the experiments of the present work in order to maximize the precision and repeatability of TMS. Specific methodology and neuronavigation systems will be discuss in the appropriate section of the individual experiments.

Nevertheless, neuronavigation systems or subject's individual MRIs might not be accessible or possible in every research facility or experiment. In that case, there are some recommendations for a comprehensive, reliable and easy way of accomplishing the search of motor hot spot. The basic protocol for hotspot search when neuronavigation is not available includes starting the search at approximately coordinates C3/C4 of the International 10/20 system for EEG electrode placement (Herwig, Satrapi, & Schonfeldt-Lecuona, 2003) and move from there to nearby scalp locations creating a grid of search over the motor cortex.

Even though, neuronavigation was used in all the experiments included in this thesis, obtaining the subjects' individual MRIs was not feasible in some of the studies. In those research protocols, we utilized a general MNI template – a detailed description of the studies that included the individual MRI and in which we used the template will be incorporated in the specific methods section. When an MNI template was used, the participants were asked to wear a swimming cap to mark head landmarks. The measures for head landmarks and the procedure to start the search of the hotspot for a figure-of-eight coil was as follows (See ***Figure 4.2*** for schematic representation of the procedure). First, we found scalp vertex by measuring inion-to-nasion and tragus-to-tragus distances, the intersection of both measurements is the vertex or Cz in the International 10/20 system (marked as an A in ***Figure 4.2A*****)**. From the vertex then we located two points at 5 cm, one towards the nasion (named as B2 in ***Figure 4.2A***) and the second towards the ear on whichever side was be stimulated – the latter is the starting point and the approximately location of coordinate C3/C4 (left/right hemispheres respectively and named as B1 in ***Figure 4.2A***); by drawing a dotted line between these two points one can help orient the coil in the optimal 45°

angle for motor cortex stimulation. In order to place the center of the coil over C3/C4 to start the search, the most common procedure is to measure from the center of the coil to the front and mark that distance over the dotted line beginning measuring from C3/C4 (point C of ***Figure 4.2A***).

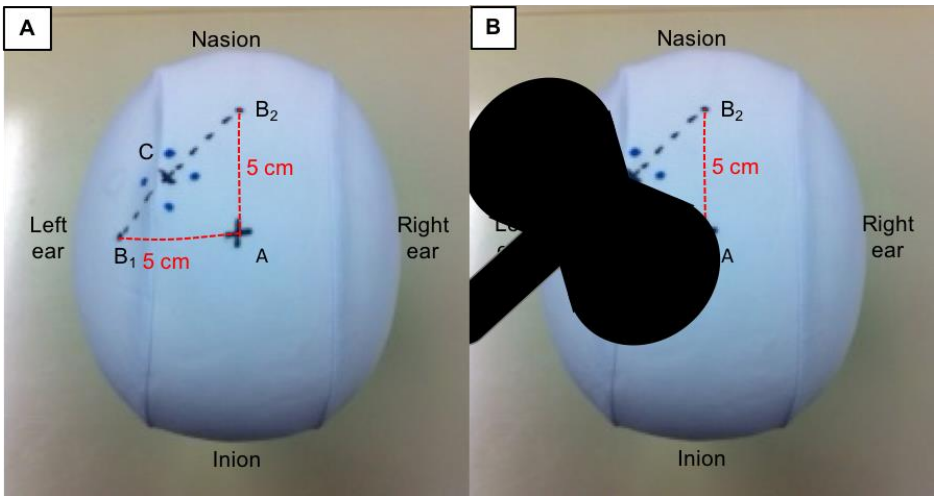

Figure 4.2. Hotspot search starting location without neuronavigation.
Figure 4.2A represents the axial view of a head with a swimming cap with the drawings of the procedure for the search of motor hotspot. A, vertex or Cz; B, 5cm spots towards the nasion and the left ear; $B_{1,}$ is approximately C3; C, is the position of the front of the coil. Blue dots show a grid that could possibly be used during the search. **Figure 4.2B** presents the coil position in relation to the scalp and the landmarks for the hand cortical representation on the left hemisphere.

After placing the coil on the correct spot in the scalp (see ***Figure 4.2B*** or ***Figure 4.1B*** *if nTMS is used*), first we started with subthreshold intensities. Intensities were then increased until the point where responses started being elicited in a reproducible way. After, we tried different spots following a grid similar to the one formed by the blue dots in ***Figure 4.2A***, we then chose the one with larger and more consistent responses and selected that as our hot spot. Once the hotspot was determined we proceeded with the search of RMT.

4.1.2 Motor thresholds

Motor threshold is defined as the lowest intensity that elicits a MEP in a target muscle either at rest (resting motor threshold, RMT) or during a certain voluntary contraction (active motor threshold, AMT). Both thresholds reflect the activation of a core of cortical elements that send spinal projections down to the target muscle (Hallett, 2007). In the case of AMT, the muscle pre-activation results in cortical but also spinal cord cells that are closer to firing, this means AMT has greater influence of the spinal cord components and is lower than RMT. Motor thresholds contribute to find an individual reference of the subject's corticalspinal excitability at the moment of the experiment and provides with a value that can be used to set the appropriate intensity of subsequent stimulations. Thresholds are usually expressed as a percentage of the MSO of a particular device.

The RMT, as defined in guidelines (Groppa et al., 2012; Rossini et al., 2015), is calculated as the lowest intensity of stimulation that elicits an MEP of at least 50 microvolts (μV) (or a visible twitch if electromyography is not used) in at least 50% of trials, commonly five of ten pulses delivered to the same region.

RMT is conditioned by the axonal excitability of corticospinal cells that is regulated by voltage-dependent Na+ channels. Several studies have shown that Na+ channel blockers, like some antiepileptic drugs (e.g. Carbamazepine), increase the RMT, in other words, these drugs decrease corticospinal excitability (for review see Paulus et al., 2008; Ziemann et al., 2015). Other factors that may have an effect on RMT are the waveforms and current directions of the TMS pulse. $Mono_{PA}$ at threshold intensities translates the activation of neural components that yield to the I1-wave. Whereas $mono_{AP}$ at that intensity, tends to elicit disperse and desynchronized late I-waves (more information about D- and I-waves is explained in *Chapter 2 – Section 2.3* of the present thesis). For biphasic waveforms, the important part of the wave at threshold intensities

seems to be the second half, thus in bi_{AP-PA}, the PA component is believed to be more relevant at low intensities (Di Lazzaro et al., 2011).

AMT has been less investigated in drug experiments. Despite the fact that, after $mono_{PA}$ stimulation and at maximum voluntary contraction, recordings from spinal cord show that the amplitude of all I-waves increase and later I-waves appear (Di Lazzaro et al., 2011), the publications cited above (Paulus et al., 2008; Ziemann et al., 2015) agreed that there is no experimental evidence to think that RMT and AMT are differentially affected by drugs. AMT was defined as the lowest intensity that elicited MEPs of at least 200 μV in at least 50% of the trials (typically 5 of 10 pulses) with the target muscle slightly contracted at around 25% of the subject's total strength.

4.2 Single-pulse protocols

4.2.1 Motor evoked potential

Over motor cortex, the application of a single pulse of sufficient intensity evokes a response of the corticospinal tract reflecting a poly-synaptic activation via layer-V PTNs. This activation leads to a muscle response or MEP that can be recorded from a target muscle by placing EMG electrodes (for further information about electromyography see *Chapter 6 – Section 6.1,* for electrode placement on ***Figure 6.1***). Latency and amplitude of the MEP are well stablished neurophysiological measures that researchers use to determine the integrity and excitability of the corticospinal tract (***Figure 4.3***). Latency is usually measured from the TMS pulse artifact to the onset of the MEP and its normal values are around 20-24 ms. MEP latency primarily translates the speed conduction of myelinated axons, and hence, it indirectly decodes the preservation of the myelin on the activated neurons. MEP amplitude is usually quantified from the negative to the

positive peaks, i.e. peak-to-peak amplitude. The amplitude of MEPs changes in a sigmoid fashion depending on the TMS pulse intensity. Input-output curves reflect these changes of amplitude in relation to intensity (see ***Figure 4.4*** for an idealized input-output curve example). Amplitude highly depends on the number of corticospinal axons that have been activated after a TMS pulse. Considering that TMS mainly excites PTNs by the activation of a complex network of intracortical circuits, the MEP amplitude is very susceptible to different brain states and cortical excitability changes of that interneuron network. Studies with CNS drugs also translate the heterogeneity of factors that may influence MEP amplitude through a complex circuitry regulated by multiple neurotransmitters (Paulus et al., 2008; Ziemann et al., 2015).

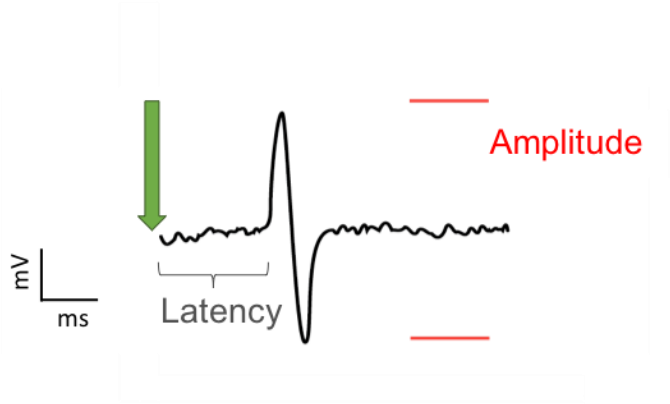

Figure 4.3. Motor evoked potential (MEP).
Latency is measured in milliseconds (ms) from the TMS pulse artifact (green arrow) to the onset of the MEP. MEP amplitude is measured in millivolts (mV) and peak to peak (difference between red lines).

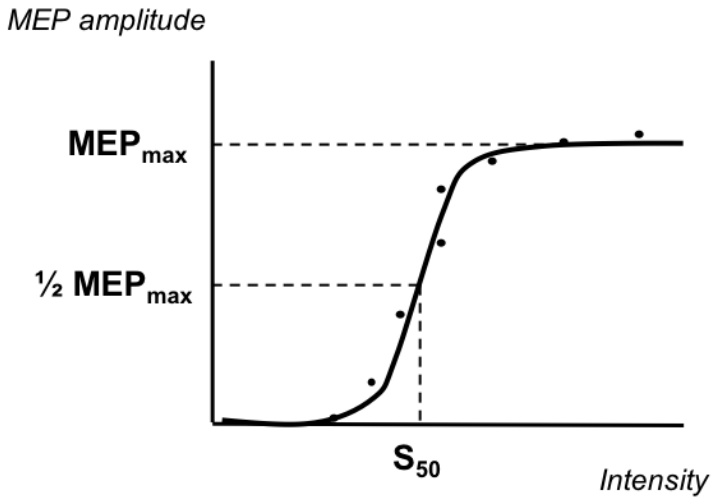

Figure 4.4. Input / Output curves (I/O curves).
I/O curves have a characteristic S-shape and are usually represented in a graph were *x*-axis corresponds to TMS intensity either as a % of maximal stimulator output (MSO) or % of motor threshold, and *y*-axis is the MEP amplitude. *Abbreviations*: MEP_{max}, maximal MEP amplitude; ½ MEP_{max}, half maximal MEP amplitude; S_{50}, intensity for ½ MEP_{max}.

4.2.2 Cortical Silent Period

A single TMS pulse at a suprathreshold intensity applied to the motor cortex during tonic contraction of a target muscle produces an facilitatory response (MEP) followed by an inhibitory effect (suppression of the maintained muscle voluntary contraction) (see ***Figure 4.5*** as an example of cSP in a healthy volunteer). Compared to a single-pulse over a relaxed muscle, the MEPs elicited during voluntary muscle activation are usually larger and have shorter latencies due to an increase in corticospinal tract excitability. This increment in cortical excitability can be measured by epidural electrodes showing and enlargement of all the I-waves while the D-wave remains intact (Di Lazzaro, Rothwell, & Capogna, 2017). The inhibitory effect of TMS is observed

as a suppression of the background EMG activity that follows the MEP. This period of EMG silence may last up to hundreds of milliseconds.

Since the exact point were the inhibitory effects of cSP starts is unknown, most of the authors quantify cSP including the active part (i.e. the MEP), therefore the measure of the cSP begins at the onset of the MEP and ends with the resumption of muscle activity (Inghilleri, Berardelli, Cruccu, & Manfredi, 1993; Orth & Rothwell, 2004). This way of measuring cSP has been called "relative cSP". However, some other publications only include the suppression of the voluntary muscle contraction ("absolute cSP").

In order to better understand the spinal cord contribution and influence on the duration of the cSP, Triggs and coworkers (Triggs et al., 1993) performed broadly used techniques that explore the integrity and excitability of the peripheral nervous system up to the spinal cord (i.e. H-reflexes and F-waves). The authors found that the first 50-75 ms of the EMG suppression are most probably due to spinal mechanisms, while the late part of the cSP is related to inhibitory processes in motor cortex. In terms of pharmacological effects, different studies have suggested that cSP is related to the GABAergic system, mainly $GABA_B$ receptor-mediator inhibitory processes. Siebner et al. (Siebner, Dressnandt, Auer, & Conrad, 1998) have reported lengthening of the cSP duration following continuous intrathecal administration of baclofen in a patient with generalized dystonia. $GABA_A$ receptor agonists also seem to prolong cSP, but overall when cSP is performed at high TMS intensities. In summary, cSP is a suitable technique to assess inhibitory processes that takes place in the whole corticospinal track as well as at a cortical level exploring GABAergic systems. In fact, cSP was found to be abnormal in some diseases that affect cortical excitability, such us Parkinson's disease (Valls-Solé, 2000), stroke (Liepert, Restemeyer, Kucinski, Zittel, & Weiller, 2005) or dystonia (Huang, Trender-Gerhard, Edwards, & Mir, 2006).

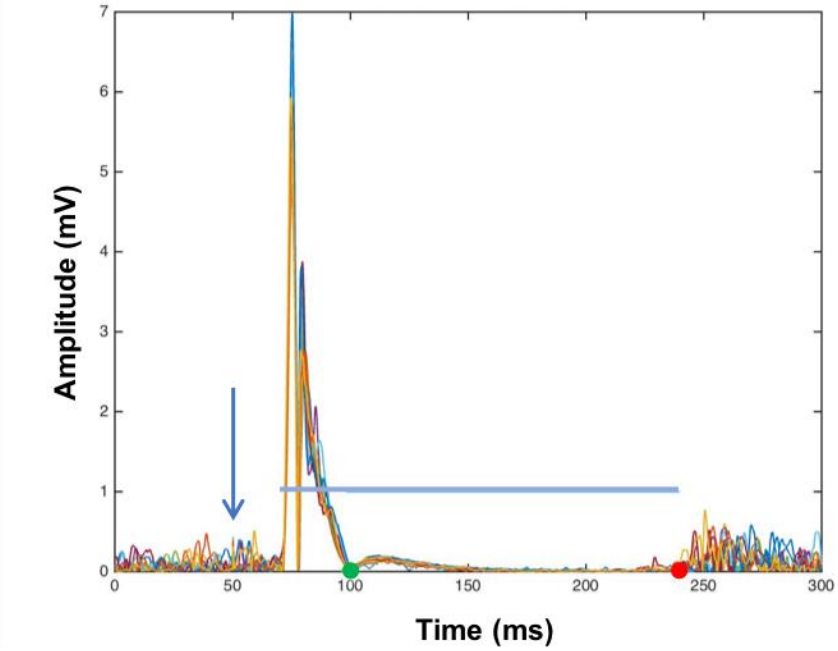

Figure 4.5. Cortical Silent period (cSP).
The figure shows ten over-imposed trials that have been rectified. The length of the cortical silent period (cSP) was measured from the onset of the MEP to the recovery of prior muscle activation (horizontal light blue line), i.e. relative cSP. The green and red dots mark how to measure the absolute cSP (from the end of the MEP to the resumption of muscle activity). The blue arrow represents the TMS pulse artifact.

4.3 Paired-pulse protocols

TMS applied in a series of two pulses or paired-pulse sequence can provide non-invasive means to evaluate the balance of inhibition and facilitation as well as cortical interaction, integrity and connectivity.

The effects of paired-pulse TMS depend on the intensity of each pulse as well as the time between pulses, the so-called interstimulus interval (ISI). The first pulse of the sequence is referred as conditioning pulse (CP) and it pre-sets the reactivity of the target cortical area. The subsequent pulse is the test pulse (TP), the pulse given at the target cortex from which we elicit a response.

Paired-pulse TMS stimulation can be delivered to a single cortical target or different cortices intra- and inter-hemispherically, to two different brain regions or by pairing peripheral and central nervous systems exploring cortical connectivity activity via circuits of more distal origins. More recently real-time EEG has been incorporated to allow the examination beyond non-motor cortical areas (Farzan et al., 2010).

Over the motor cortex, the MEP elicited by a paired-pulse TMS (conditioned MEP) is compared to unconditioned MEP (i.e. MEP elicited by a non-conditioned or single-pulse at a constant supra threshold intensity) as a % of change.

Paired-pulse TMS to primary motor cortex allows for assessing the balance between inhibitory and facilitatory effects of specific intracortical neural networks as well as the corticocortical connections in relation with GABAergic and glutamatergic systems. Different protocols have been described depending on the intensity of the pulses and the length of the ISI. These techniques had been broadly studied in the motor cortex and we will expand on their physiological connotations bellow. The paired-pulse TMS protocols most frequently reported in literature have been short-interval intracortical inhibition (SICI) (Kujirai et al., 1993), long-interval intracortical inhibition (LICI) (Valls-Solé, Pascual-Leone, Wassermann, & Hallett, 1992) and intracortical facilitation (ICF) (Kujirai et al., 1993; Valls-Solé et al., 1992), schematically illustrated in ***Figure 4.6***. Conventionally paired-pulse TMS have been performed with $mono_{PA}$ pulses, most probably due to historical reasons and technical availability when they were first described.

For the experiments of this thesis, we chose the most reported paired-pulse TMS applied to a single cortical region, primary motor cortex (i.e. SICI, LICI and ICF).

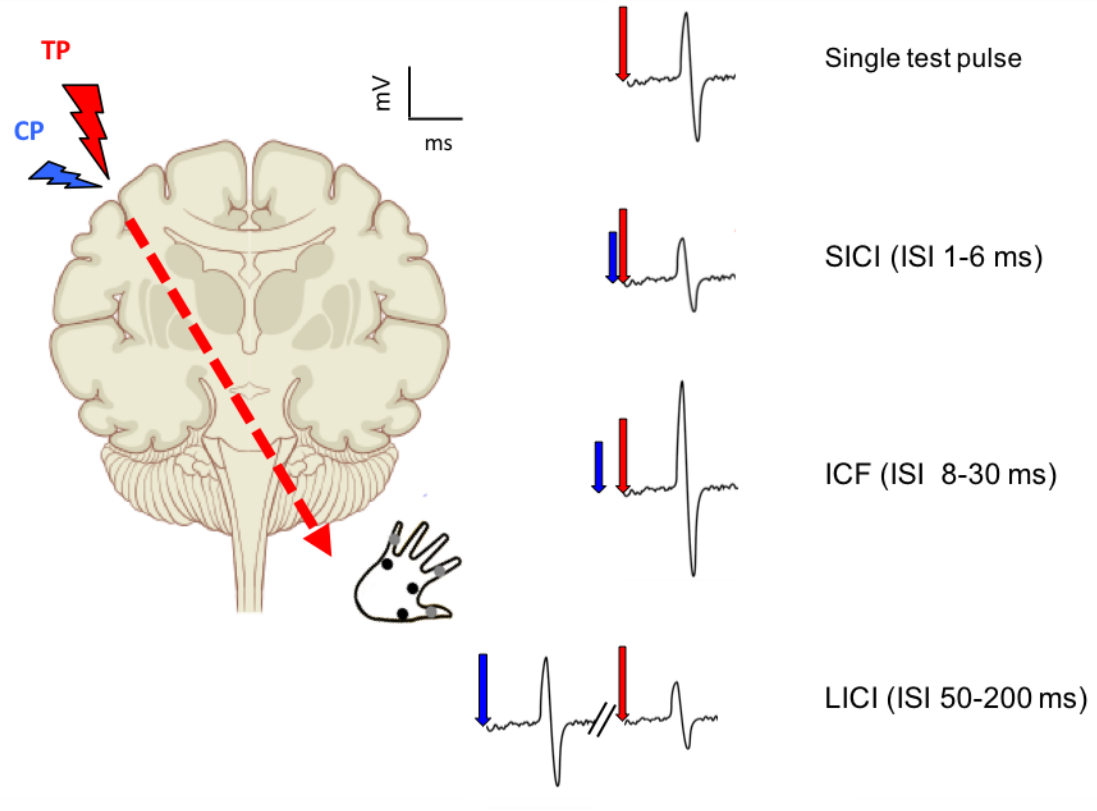

Figure 4.6. Schematic representation of single- and paired-pulse protocols.
This graph presents the responses to single-pulse TMS (unconditioned MEP) and the three different paired-pulse protocols used in this thesis. *Abbreviations*: CP, conditioning pulse (presented in blue); ICF, intracortical facilitation; ISI, interstimulus interval; LICI, long-interval intracortical inhibition; SICI, short-interval intracortical inhibition; TP, test pulse (presented in red).

4.3.1 Intracortical inhibition

During the protocols of intracortical inhibition the amplitude of the MEPs is dramatically decreased compared to a MEP elicited by a suprathreshold TMS single-pulse. Both SICI and LICI have different characteristics of pulse parameters but they also target particular intracortical and neurotransmitters systems.

SICI consists in a subthreshold CP followed by a suprathreshold TP with an ISI between 1 and 6 ms (Kujirai et al., 1993). SICI can be enhance by drugs that increase $GABA_A$ activity, therefore, it may provide information primarily about the activation of the $GABA_A$-ergic inhibitory interneurons in the cortex (Chu, Gunraj, & Chen, 2008; Di Lazzaro et al., 2000, 2006; Paulus et al., 2008; Teo, Terranova, Swayne, Greenwood, & Rothwell, 2009; Ziemann, 2013)(Chu et al., 2008). At the shorter ISIs, SICI is believed to also reflect neural refractoriness period and synaptic inhibitory processes (Cengiz, Murase, & Rothwell, 2013). In the previously described theoretical canonical model from Di Lazzaro (Di Lazzaro & Rothwell, 2014) and subsequent studies where the authors recorded D- and I-waves after TMS (see *Chapter 2 – Sections 2.1* and *2.2*), SICI was found to inhibit late I-waves (I_2 and later I-waves). D- and I_1-waves did not decrease after SICI (Nakamura et al., 1996; Di Lazzaro, Restuccia, et al., 1998; Di Lazzaro et al., 2002; Ni et al., 2011). Hence, all together SICI conditioning pulse interacts with chains of inhibitory $GABA_A$ interneurons in layers II and III of the primary motor cortex probably by producing IPSP in the circuits.

On the other hand, LICI happens when both CP and TP are supra threshold and the ISI is from 50 to 200 ms or longer (Valls-Solé et al., 1992). LICI is believed to be the product of postsynaptic $GABA_B$ receptor activity (Chu et al., 2008; Paulus et al., 2008; Werhahn, Kunesch, Noachtar, Benecke, & Classen, 1999; Ziemann, 2013). As SICI, LICI also inhibits later I-waves while D- and I_1-waves remain intact (Nakamura et al., 1996; Di Lazzaro, Restuccia, et al., 1998; Di Lazzaro et al., 2002; Ni et al., 2011). Therefore, LICI activates inhibitory circuits of $GABA_B$ interneurons in layers II and III of the motor cortex.

4.3.2 Intracortical facilitation

Facilitatory protocols enhance intracortical circuits and produce larger conditioned MEPs when compared to unconditioned MEPs. The most frequently used facilitatory paired-pulse TMS

is called ICF. ICF is elicited when the CP is subthreshold followed by a suprathreshold TP. ISI is from 8 to 30 ms (Kujirai et al., 1993; Valls-Solé et al., 1992; Ziemann, Rothwell, & Ridding, 1996). Even though the exact mechanisms remain unclear, it is largely believed that ICF indicates an excitatory neurotransmission mediated by NMDA receptor. This is supported by pharmacological studies, which showed a decrease of facilitation by NMDA receptor antagonists (e.g. dextromethorphan or memantine) (Paulus et al., 2008; Sohn YH, Jung HY, Kaelin-Lang A, & Hallett M, 2002; Ziemann, 2013; Ziemann, Tam, Butefisch, & Cohen, 2002). Unlike inhibitory protocols, ICF does not change the amplitude or number of corticospinal waves (Di Lazzaro et al., 2006; Ni et al., 2011). Different hypotheses have been proposed, first ICF may result from the recruitment of circuits unrelated to those involved in I-wave generation evoking a more desynchronized activity not evident in the epidural records. Additionally, despite ICF cortical origin (Cash et al., 2017), contributions from other cortices or brain structures as well as of spinal mechanisms cannot be entirely excluded (Di Lazzaro et al., 2006). Following this last assumption Wiegel et al. (Wiegel, Niemann, Rothwell, & Leukel, 2018) have studied the possible subcortical contribution to ICF by pairing the ICF protocol, and the sub- and suprathreshold TMS pulses to H-reflexes. The H-reflexes evaluate the spinal cord excitability by eliciting reflex mechanisms after the electrical stimulation of peripheral sensory nerve fibers. By pairing those two protocols the authors were able to objectify the modulation of the H-reflex response after a subthreshold TMS pulse. This observed facilitation in the H-reflex is related to its facilitation after the ICF protocol suggesting that the subthreshold CP is able to trigger subcortical and spinal processes that may contribute to the facilitation of MEPs.

4.4 Repetitive Transcranial Magnetic Stimulation

Repetitive transcranial magnetic stimulation (rTMS) refers to a series of TMS pulses applied in form of trains with a specific internal frequency. Depending on the particular frequency

of rTMS, dynamics of human neural networks can be modified beyond the duration of the stimulation by modulating those networks in a selective and maintained way. This repetitive form of TMS enables the characterization of the mechanisms of cortical plasticity and has become a treatment tool of great value (for review see Rotenberg, Horvath, & Pascual-Leone, 2014; Wassermann et al., 2008).

Nowadays, several rTMS protocols have been described. These rTMS protocols have been historically classified into inhibitory or facilitatory depending on their ability to either enhance or suppress brain excitability. Among the most frequent rTMS presentations we should point out low-frequency rTMS (frequencies equal or under 1Hz, this is a pulse per second), high-frequency rTMS (frequencies equal or over 5Hz) and more complex or patterned rTMS paradigms that follow intrinsic brain oscillatory activity, like TBS protocols. One of the experiments of the present thesis explores the reliability of a specific TBS protocol (i.e. iTBS). iTBS, when evaluated at a population level, enhances cortical excitability. The following section will be dedicated to explain TBS and its physiological implications.

4.4.1 Theta-burst stimulation

There is an increasing interest in directly measuring the mechanisms of human brain plasticity by the use of TMS. In *Chapter 2 – Section 2.4* we defined plasticity as the ability of the CNS to adapt to different internal and external conditions. At a synaptic level, plasticity results from the enhancement or diminution of the strength of the synaptic transmission (i.e. LTP and LTD, respectively). In animal models, LTP and LTD have been long ago explored by the application of a repetitive pattern of electrical stimuli on rodent hippocampal slices (Bliss & Lomo, 1973; Diamond, Dunwiddie, & Rose, 1988; Bliss & Cooke, 2011). ***Figure 4.7*** represents the effects of high- and low-frequency stimulation on intracellular recordings. As shown in the figure,

high frequencies lead to a potentiation of the EPSP and thus to LTP, while on the contrary, low frequencies yielded inhibitory responses and LTD.

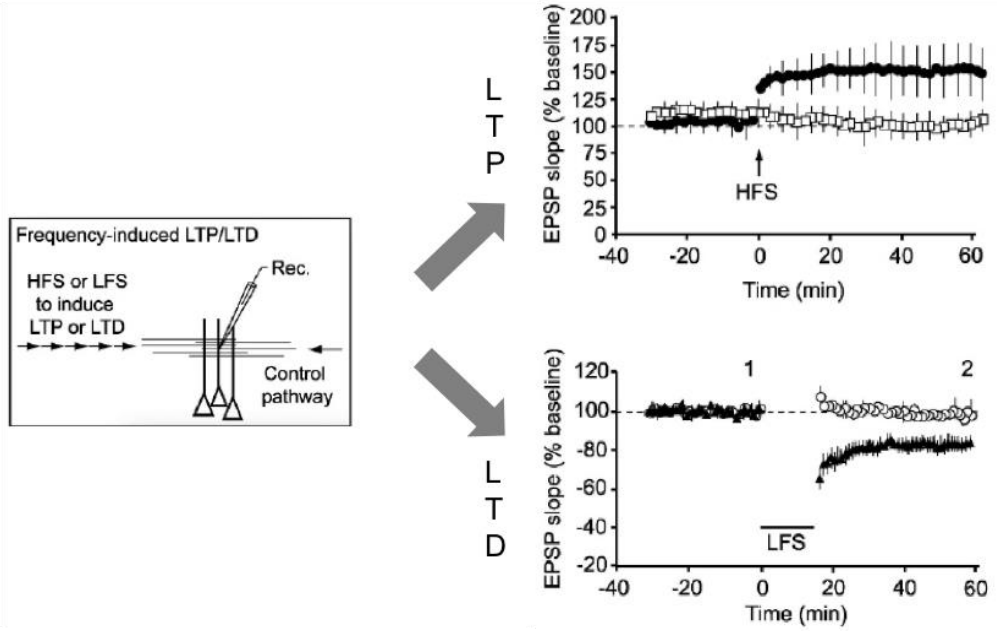

Figure 4.7. Long-term potentiation and depression results in animal model studies.
Schematic representation long-term potentiation (LTP) and depression (LTD) induction in a pathway after high- and low-frequency stimulation (HFS and LFS, respectively). Upper- and lower-right diagrams present the results on the excitatory post-synaptic potentials (EPSP) after HFS and LFS compared to controls showing LTP and LTD responses. Adapted from Bliss & Cooke, 2011.

Following the approach of the animals studies and *in vitro* preparations, an ultra-high frequency patterned rTMS application termed TBS emerged for human use (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2004). TBS induces a long-lasting neuromodulation outlasting the stimulation time with shorter stimulation durations than standard rTMS. TBS is named after the intrinsic basic frequency of the protocol that resembles the theta range of EEG recordings (5-8 Hz). The basic structure of TBS involves bursts of 3 pulses at a 50 Hz frequency repeated every

200 ms (i.e. 5 Hz). Similar to low and high frequency rTMS, TBS can be applied continuously during 40 sec (600 pulses in total) reducing the MEP amplitude in a LTD-like effect for about 50-60 min, or intermittently (2 sec on, 8 sec off) for 190 sec to achieve an LTP-like effect increasing MEP amplitude during 60 min after the stimulation (Wischnewski & Schutter, 2015).

These TBS protocols have been used to identify age-related changes in the mechanisms of plasticity across the lifespan in healthy individuals (Freitas et al., 2011), and of particular importance for the present thesis, on T2DM (Fried et al., 2017), and AD (Di Lorenzo et al., 2016; Koch et al., 2012); among other disorders where TBS revealed altered neuroplastic mechanisms such as autism spectrum disorders (Oberman et al., 2012), traumatic brain injury (Tremblay et al., 2015) and schizophrenia (McClintock et al., 2011).

Freitas et al. (Freitas et al., 2011) showed that the inhibitory effect and the time-to-baseline of the LTD-like responses after cTBS decrease with advancing age suggesting a progressive physiological decline of plasticity cortical processes. In disorders associated with aging, such as T2DM and AD, it has been shown a reduction of the LTP-like response after iTBS (Koch et al., 2012; Di Lorenzo et al., 2016; Fried et al., 2017) when compared to controls. In particular, the decrease of LTP-like effects after iTBS in AD may yield absent responses and, even though the impairment of the response is not related to the years from the onset of the disease, AD patients presenting more altered LTP-like plasticity have more severe cognitive decline (Di Lorenzo et al., 2016). Interestingly enough, AD patients showed LTP-like impaired plasticity but preserved or more prominent LTD-like effects after cTBS. The comparison between T2DM or AD patients to controls is shown in ***Figure 4.8***.

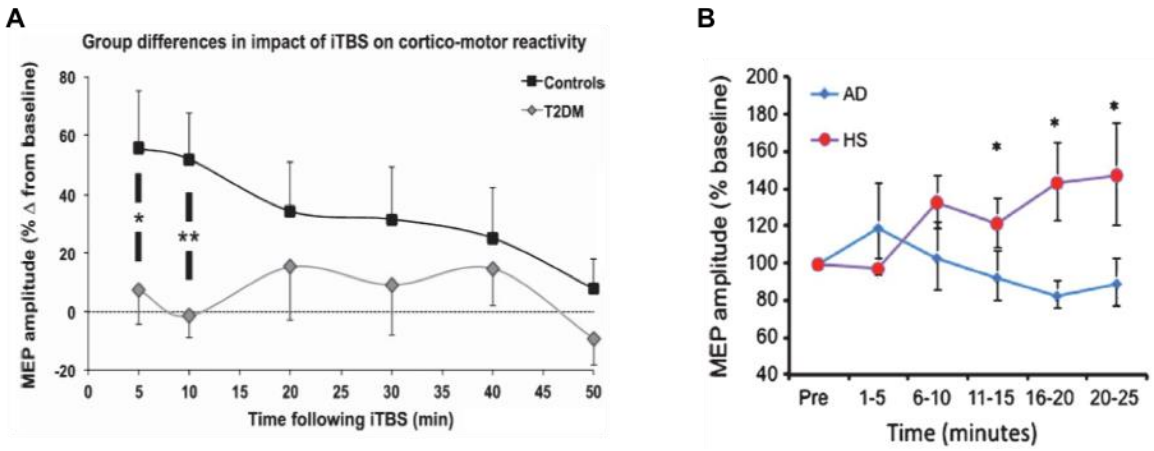

Figure 4.8. Effects of iTBS on T2DM and AD patients compared to healthy controls.
The figure represents the effects of iTBS, known to induce LTP-like plasticity, in patient population and age-matched healthy controls. (**A**) Shows the comparison of the TMS-plasticity measure between Type-2 Diabetes Mellitus (T2DM) patients and healthy controls (Fried et al., 2017) where at time 5 and 10 post-iTBS the authors showed a significant difference in MEP amplitude enhancement. (**B**) Compares the LTP-like effects on patients with dementia due to Alzheimer's Disease (AD) and healthy controls (Koch et al., 2012). AD and controls were significant different after 10 min post-iTBS, this study also showed a paradoxical inhibitory response in the AD group.

Thus, in the near future, TBS as a neuromodulation tool may have a relevant role in the diagnosis and prognosis of highly prevalent debilitating diseases associated with increasing age such as AD and T2DM. While the effects of TBS on these populations have been tested, the reliability of TBS and possible influencing factors has been insufficiently studied (see *Chapter 2 – Section 2.5* for further information). Consequently, as the popularity of TBS increases, the study of the factors that may have an impact on the inter- and intra-subject variability of TBS becomes essential.

5 Transcranial Static Magnetic Stimulation

Static magnetic fields have been found to modify the excitability of the cortical areas subjacent to the point of stimulation as explained in *Chapter 2 - Section 3*. For the experiments of the present work we stimulated the primary motor cortex with a cylindrical neodymium magnet (3.8 cm diameter x 3.8 cm height), (NdFeB; 45 MGOe; megagauss-oersteds, nominal strength 65 kg ≈ 0.5 tesla-T (Model DX8X8 K&J Magnetics, US)) during 15 minutes. A non-magnetic replica of identical appearance and weight (i.e., indistinguishable from the magnet) was used for sham tSMS. Even though different experiments have shown that the polarity of the magnet do not influence the effects over motor cortex, we decided to use south polarity (marked with an “S” in both the real and the sham cylinders) as it has been commonly reported in previous studies (Oliviero et al., 2011; Silbert et al., 2013; Nojima et al., 2015). Further details on the specific features of the methodology are described in *Chapter 10*.

Magnetic fields, unlike electric fields, can go through tissues without changing or loosing intensity, however all magnetic fields decrease exponentially with the distance. Therefore, knowing that the motor cortex might be few centimeters (~2-3 cm) far away from the stimulation point over the scalp, the amount of the magnetic flux that effectively stimulates the motor cortex is not the same as the nominal strength of the cylindrical neodymium magnet. Rivadulla and colleagues (Rivadulla et al., 2014) characterized the decay of the magnetic field (B-field) and the reproducibility of the magnet they used in their previous experiments (Oliviero et al., 2011; Silbert et al., 2013; Kirimoto et al., 2014). The authors showed that the size of the magnet is relevant for cortical stimulation given that at 2-3 cm (calculated distance of motor cortex from the magnet on the scalp) the B-field strength is about 120-200mT. More recently, Tharayil et al. (Tharayil et al., 2017) have modelled the B-field into a realistic 3-D human head adding the magnetic properties

of the various tissues. This modelling concluded that the most important factor for the stimulation with SMF was the cortex-to-magnet distance and that the strongest B-field was around the edges of the magnet.

Similarly, we have measured the decay of the B-field of the magnet we used in our experiments and found a comparable decrease with distance and analogous intensities for the cortex at the calculated distance (***Figure 5.1***).

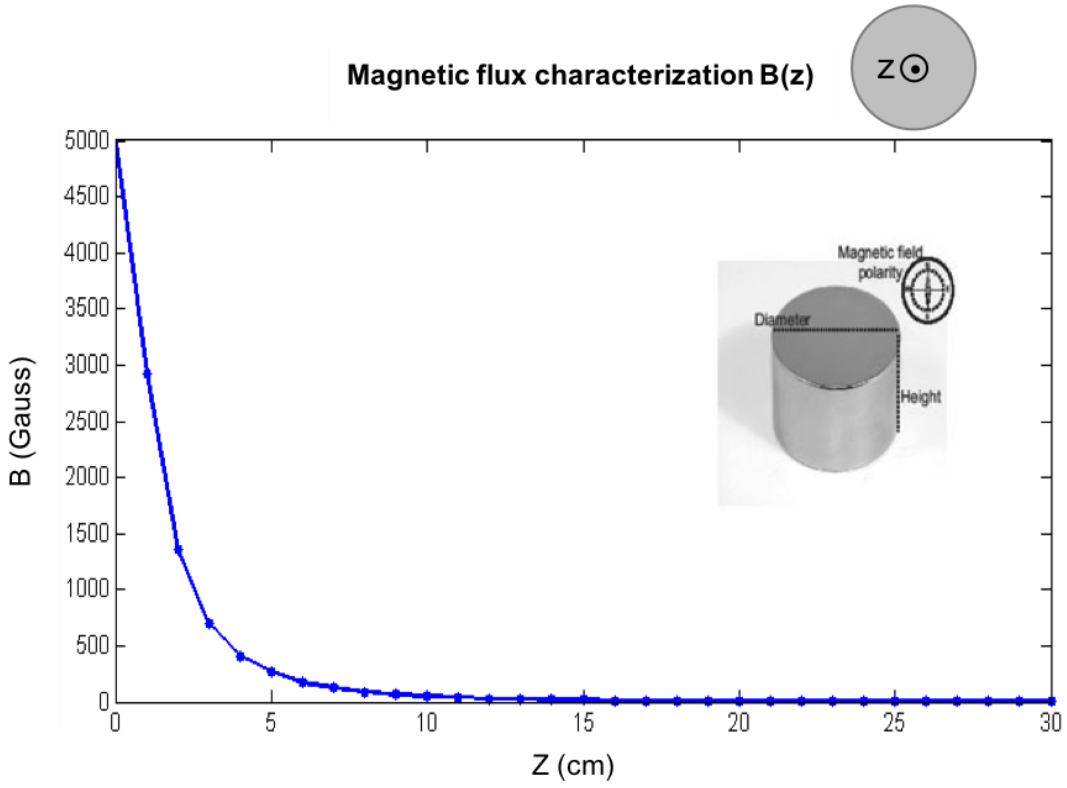

Figure 5.1. Characterization of the magnetic flux (B) and its exponential decay with the distance measured from the center of one of the poles (Z).
Y-axis presents the magnetic flux (B) in Gauss (1T = 10000 Gauss) and the x-axis shows the distance from the center of the magnet (Z) in cm. At 2cm, B is over 1200 Gauss (120 mT).

6 Neurophysiological recording techniques

The utility of the NIBS techniques can be greatly enhanced by combining stimulation with neurophysiological and imaging techniques that allow the objective measurement of the effects of magnetic fields on brain's reactivity and plasticity and can guide us on where and how to stimulate. In the present thesis two of the most widely known neurophysiological techniques (i.e. EMG and EEG) have been used to evaluate the effects of different factors in TMS reliability (experiments of *Chapters 7*, *8* and *9*) but also to explore the effects of tSMS on motor cortex excitability (experiments of *Chapter 10*).

6.1 Electromyography

During the studies of the present thesis we performed surface electromyographic recordings of the intrinsic hand muscles using standard cleaning procedures and the belly-tendon montages recommended by international guidelines (Deuschl & Eisen, 1999). This means that the area where the electrodes were attached was scrubbed with alcohol swipes for skin cleaning and preparation. The electrodes were positioned as shown in ***Figure 6.1*** following a conventional belly-tendon montage, with the negative electrode (or active electrode) over the belly of the first dorsal interosseous (FDI) muscle and the positive electrode (or reference) over first interphalangeal joint of the second finger. The ground electrode was placed over the ipsilateral ulnar styloid process. Specific features of the montages, targeted muscles, EMG devices, filter characteristics, pre- and post-processing methods of the recordings will be described in the appropriate methods section of the different experiments.

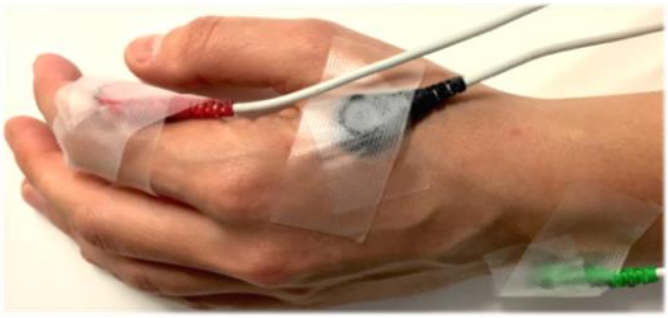

Figure 6.1. Standard belly-tendon montage for first dorsal interosseous (FDI) muscle.
The positive or reference electrode is presented in red and positioned over the proximal interphalangeal joint. The black electrode represents the negative or active electrode and it is over the belly of the muscle or the motor point. The ground electrode is over the ulnar styloid in green.

Electromyography is a wide-world used technique that detects biological potentials generated by an electric volley that activate cells of the central and peripheral nervous systems. Therefore, EMG can be a very useful technique for the evaluation and monitoring of the somatosensory and motor systems both from a clinical and a research perspective. In the case of TMS, EMG can detect the electrical volley that comes from the direct or indirect activation of pyramidal tract neurons within the motor cortex. That motor volley travels all the way down through the spinal cord and finally activates the muscle, evaluating the integrity of the corticospinal tract pathway. Throughout the present work, EMG is used to evaluate the changes in motor cortex and spinal tract reactivity and excitability after the exposure to either static or electromagnetic fields and the reliability of different TMS protocols.

6.2 Electroencephalography

Understanding and defining neural networks and their dynamics has become essential to disentangle intrinsic brain communication mechanisms. Neural networks translate interactions between different brain regions and can explain cognitive and behavioral processes. The information transfer in those networks within and across brain regions is believed to occur through synchronized oscillatory activity (Bonnefond, Kastner, & Jensen, 2017; Buzsaki, 2005; Fries, 2005, 2015; VanRullen, 2016). Furthermore, abnormal or desynchronized oscillatory dynamics, defective interactions or damaged neural network may lead to neurological and psychiatric disorders.

EEG is a direct and non-invasive way to measure the spontaneous and event-related electrical activity generated in the convexity of the CNS. It is an exceptional multidimensional tool for studying cerebral electrophysiology and neurocognitive processes given its high-temporal resolution. This neurophysiological technique can capture neural signals and brain synchronized oscillatory dynamics during the timeframe they are happening. EEG directly measures the oscillations which translate biophysical changes at a neural population level. Despite the high-temporal resolution of EEG, its ability of spatial localization is limited. EEG allows to discriminate between changes in brain oscillations at a large scale (e.g. changes in synchronization between brain areas of a given network) but it is not very accurate answering questions about specific small areas or areas far deep from the cortex.

Neural oscillations have been conventionally categorized into five frequency bands, which are: delta (0.5**–** 3.5 Hz), theta (4**–** 7 Hz), alpha (8**–** 12 Hz), beta (13**–** 30 Hz) and gamma (**>** 30 Hz). The study of brain oscillatory activity is of great relevance in defining those neural networks and understanding brain processes and cognition. Nevertheless, the direct relationship between frequency bands and cognitive or behavioral processes remains unsolved.

Of special relevance for the present thesis is the role of the most important frequency bands in terms of behavioral response of the motor cortex. The functional interpretation of changes in alpha and beta bands as part of the cortical control of the motor system has been largely discussed. The alpha band is believed to be more related to a phasic inhibition (i.e. sudden stop of a initiated movement or a suppression of the movement), in other words, a widespread general break of the system (Pani et al., 2014). However, what the beta range represents in the motor cortex is less well understood.

The role of beta band has been largely discussed during the last decades. While before, most of the scientific community argued that beta was a resting rhythm, nowadays the latest investigations have suggested a more intricate relationship. Beta band most probably represents the level of motor preparation and is a rhythm of movement prevention. This hypothesis was well stablished in different studies were spontaneous (Gilbertson et al., 2005) or entrained (Pogosyan, Gaynor, Eusebio, & Brown, 2009) beta activity was highly associated to worse or slowed motor performance. This relationship is also supported by the findings of critically enhance beta oscillations in diseases affecting the motor system such as Parkinson's Disease (Little & Brown, 2014) and a casual relation between this enhancement and akinetic and dyskinetic symptoms.

In a recent review, Engel and Fries (Engel & Fries, 2010) have also suggested that the main role of the beta band is the maintenance of the status quo in the motor system, and not only the lack of movement or the prevention of it, reflecting a fine regulation of motor behavior.

Knowing that EEG recordings can provide a vast amount of physiological information, combining NIBS techniques and EEG will allow deeper understanding of the brain and its physiological changes, as well as the pathophysiological processes of various diseases. As an example, a pulse or a train of TMS pulses over a specific brain area changes immediately the oscillatory properties of region under the coil but will be followed by a spread to areas that are anatomically but also functionally connected, inducing the modulation of neural networks that connect cortical areas with subcortical regions and also connect both hemispheres. Furthermore,

NIBS-EEG studies will facilitate the elucidation of brain behavior in non-motor areas through objective and quantifiable tools.

FIRST BLOCK OF EXPERIMENTS: RELIABILITY OF TRANSCRANIAL MAGNETIC STIMULATION AND INFLUENCING FACTORS.

Test-retest reliability refers to the study of the consistency of the outcome of a given technique regardless how many times you perform it o who performs it. The study of the reliability of TMS, and notably the study of the factors that may influence it, can help to better understand the TMS-brain interaction and the cortical processes that take place following a TMS pulse, but more importantly, it will improve the overall performance of TMS for future studies and for eventual clinical applications.

During this first set of experiments, we evaluated factors that we hypothesized would influence the reliability of the TMS outcome after different single-, paired-pulse and repetitive TMS protocols. As already mentioned in the general introduction to the current state of the NIBS field of the present thesis (*State of the Art* (*Chapters 2* and *3*)), these factors can be categorized into physical – technical (i.e. waveform and current direction) and physiological (i.e. ageing, age-related and metabolic diseases).

The first of these group of experiments analyzes the so called physical or technical factors in a sample of young and healthy controls (*Chapter 7*). In other words, those factors that we can control by introducing certain parameters in the setup of our devices. More specifically, our work was focused on the influence of waveform (biphasic vs monophasic) and current direction (PA vs AP) based on previous publications which argued that these TMS pulse parameters activate

rather specific neural populations. In a second experiment, we evaluated the impact of aging and of the two more prevalent age-related and metabolic diseases, i.e. dementia due to Alzheimer's (AD) and Type-2 Diabetes Mellitus (T2DM), on single- and paired-pulse TMS as well as a particular rTMS protocol that is well-known to allow the assessment of cortical plasticity (i.e. iTBS) (*Chapter 8*). Finally, we compared the outcomes of the single- and paired-pulse TMS protocols between the young and older healthy controls for those waveforms and current directions that were common between the two experiments to discern is age as a factor has any influence (*Chapter 9*).

7 The effects of waveform and current direction on the efficacy and test-retest reliability of transcranial magnetic stimulation

7.1 Introduction

Despite the substantially growth on the use of TMS, there is still an important lack of understanding the underlying mechanisms, especially with regard to the interaction of TMS with the neural substrate, and how different parameters influence the efficacy and reliability of TMS-based neurophysiological assessments. In *Chapter 2* of the present thesis we described some of the parameters that are known to influence the current-brain interaction. Although we know some of these parameters may have an impact on the effects of TMS, a deeper understanding of these issues is crucial to assess the utility of TMS measures as possible neurophysiologic biomarkers in health and disease.

The seemingly straightforward account of the mechanisms of TMS belies the complex interplay between the various physical parameters and configurations of the induced current and

the properties of the individual's brain where the electro-magnetic induction takes place. In particular, factors such as pulse waveform and current direction have not received sufficient attention in the literature, despite clear evidence of their importance in shaping the outcome of TMS (Mills et al., 1992; Sakai et al., 1997; Kammer et al., 2001). Most TMS stimulators generate pulse waveforms that are either biphasic or monophasic (although other shapes such as half-sine and square-wave pulses are also available on some machines). These two types of waveforms can be distinguished based on the length and duration of the first and second phases of the pulse waveform (see *Chapter 2 – Sections 2.2* and *2.3* for further information on the distinctive features of pulse parameters).

In addition to pulse shape, the direction of the induced current in the brain is determined by the type of coil (e.g., circular or figure-8), the direction of the current through the coil windings (e.g., posterior-to-anterior or anterior-to-posterior at the center of a figure-8 coil), the orientation of the coil relative to the stimulated cortex (e.g., perpendicular to motor cortex, which is approximately a 45° angle relative to the midline), and sulcal geometry (Salvador et al., 2011).

Previous studies suggest that specific waveforms and current directions preferentially stimulate different neural components in different cortical layers. These studies are based on invasive epidural recordings of the efferent corticospinal (Di Lazzaro, Oliviero, Mazzone, et al., 2001; Di Lazzaro et al., 2003, 2011; Di Lazzaro & Rothwell, 2014). A corticospinal volley elicited by TMS can be composed of D- and/or I-waves, that translate the direct or indirect activation of the PTNs, respectively (Amassian, Cracco, & Maccabee, 1989; Amassian, Quirk, & Stewart, 1990; Thompson et al., 1991; Burke et al., 1993). In *Chapter 2 – Section 2.3* we described these studies and their implications in depth. Based on these studies, different theoretical canonical cortical models have been proposed in order to better explain the current-brain interaction (Ziemann & Rothwell, 2000; Di Lazzaro & Rothwell, 2014; Rusu et al., 2014).

As with any technique, the outcome of TMS can be assessed in terms of its efficacy and consistency. In other words, will a given TMS protocol produce the desired (or expected) outcome,

and is this effect reproducible when assessed in the same subjects on different occasions? Both of these questions are increasingly relevant as TMS-based neurophysiological measures are explored for their diagnostic and prognostic potential. While several studies have examined the effects of pulse waveform and current directions on TMS measures (Mills et al., 1992; Sakai et al., 1997; Niehaus, Meyer, & Weyh, 2000; Kammer et al., 2001; Orth & Rothwell, 2004; Takahashi et al., 2005; Sommer et al., 2006, 2013; Ni et al., 2011; Delvendahl, Gattinger, et al., 2014; Delvendahl, Lindemann, et al., 2014; D'Ostilio et al., 2016; Stephani, Paulus, & Sommer, 2016), no study, to our knowledge, has investigated the influence of these parameters on both the efficacy and test-retest reliability of several TMS measures including paired-pulse protocols. The present study aims to fill this gap through a direct comparison of three widely used TMS pulse configurations in the most common single- and paired-pulse TMS measures obtained from healthy individuals in two sessions.

7.2 Methods

7.2.1 Participants

Twenty-six healthy adults (age range, 18–35 years, 14 females, 22 right-handed) were enrolled in the study.

All participants completed two identical TMS sessions (intersession interval range 1–70 days; median = 10.5 days). During both sessions, all participants underwent two TMS safety forms to screen for possible contraindications (see appendix C) and side effects (see appendix E). These TMS safety screening forms were based on the safety guidelines for TMS by The Safety of TMS Consensus Group (Rossi et al., 2009), for more information read *Chapter 2 – Section 2.6* of the present thesis. Handedness was determined in the first visit by revised Edinburgh

Handedness Inventory (Oldfield, 1971) (see appendix B). Participants were randomly separated into three groups, which differed only in the pulse waveform characteristics: nine subjects received $mono_{PA}$, seven received $mono_{AP}$, and seven subjects received $bi_{AP\text{-}PA}$ stimulation. All the current directions above are indicated in their relation to motor cortex. One participant from $mono_{PA}$ condition was excluded from all analyses because of a prior history of traumatic brain injury that was not disclosed during enrollment/screening. Two other participants from $mono_{AP}$ group were excluded from the study because their RMT was higher than 83% of MSO and, therefore, the stimulation at 120% of RMT was not feasible. None of the remaining participants had a history of medical disease or contraindication to TMS (questionnaires of appendix A and C screened for any medical records and/or possible TMS contraindications), and all of them had normal physical and neurological examinations. Participants' demographics are presented in ***Table 7.1***.

7.2.2 Electromyography

Surface EMG activity was recorded from the dominant hand's FDI using a PowerLab 4/25T data acquisition device and Scope software (ADInstruments, Colorado Springs, CO, USA). As mentioned in *Chapter 6 – Section 6.1*, electrodes were placed over the FDI in a belly-tendon montage. EMG data were digitized at 1 kHz for 250 ms following each stimulus trigger and amplified with a range of ±10 mV (band-pass filter 0.3–1000 Hz). During the silent period trials, live EMG was monitored throughout the protocol to provide feedback for continuous muscle contraction. MEP peak-to-peak amplitudes (mV) of the non-rectified signal and silent period duration (ms) were recorded for individual traces. Participants were also monitored for drowsiness and asked to keep their eyes open throughout the experiment.

Table 7.1. Participants characteristics.

Participant	Waveform/ Current direction	Gender	Handedness	Medications	Difference between visits	
					Days	Start-time (h)
1	bi_{AP-PA}	Male	Right	–	1	0.5
2	bi_{AP-PA}	Female	Right	birth control	25	0.5
3	bi_{AP-PA}	Male	Right	–	1	1
4	bi_{AP-PA}	Female	Right	–	5	3
5	bi_{AP-PA}	Male	Right	–	7	0
6	bi_{AP-PA}	Male	Left	–	7	0
7	bi_{AP-PA}	Male	Right	–	5	0
8	$mono_{AP}$	Female	Right	birth control	36	0
9	$mono_{AP}$	Male	Right	–	5	1
10	$mono_{AP}$	Female	Right	–	24	5
11	$mono_{AP}$	Female	Right	birth control	11	2
12	$mono_{AP}$	Female	Left	birth control, cetirizine hydrochloride	36	2
13	$mono_{AP}$	Female	Right	birth control, vitamins	70	0.5
14	$mono_{AP}$	Female	Right	–	13	0
15	$mono_{PA}$	Male	Right	–	16	4.5
16	$mono_{PA}$	Female	Right	birth control	16	0
17	$mono_{PA}$	Male	Right	vitamins	11	1
18	$mono_{PA}$	Female	Right	birth control	4	0
19	$mono_{PA}$	Female	Right	birth control	7	0
20	$mono_{PA}$	Male	Left	–	10	0.25
21	$mono_{PA}$	Female	Right	–	12	5
22	$mono_{PA}$	Female	Right	birth control	14	3
23	$mono_{PA}$	Male	Left	cetirizine hydrochloride	9	2

Abbreviations: biAP-PA, biphasic anterior-posterior—posterior-anterior; monoAP, monophasic anterior-posterior; monoPA, monophasic posterior-anterior.

7.2.3 Transcranial Magnetic Stimulation

Each TMS visit was performed by one of three experienced TMS technicians and the same technician performed both visits for a given subject. Participants were comfortably seated in a chair with their arms rested in a natural ~90º angle on a table in front of them. nTMS was performed with a MagPro X100 device using a hand-held Cool-B65 figure-of-eight coil (outer diameter 75mm) placed over the primary motor cortex in the dominant hemisphere with the handle pointing backwards and at an angle of 45º (MagVenture A/S, Denmark). MagPro X100 devices allow to change between waveforms and current directions without needing to change the coil position. A Polaris infrared-optical tracking system (Northern Digital Inc., Waterloo, ON, Canada) and a Brainsight TMS neuronavigation system (Rogue Research, Inc., Montreal, QC, Canada) with a brain MRI template was used to ensure consistent targeting throughout the experiment.

Each session began by assessing the motor cortex "hotspot" and RMT for FDI muscle. The hotspot was identified *de novo* at each visit, using the C3/C4 method described in *Chapter 4 – Section 4.1*, and designated as the neuronavigation target for the remainder of the visit. RMT was calculated following the approach we previously described in the general methodology (*Chapter 4 – Section 4.1*) and that has been defined by the International Federation of Clinical Neurophysiology guidelines (Rossi et al., 2009; Rossini et al., 2015).

After assessing RMT, a battery of standard TMS neurophysiological measures were acquired in the following order: baseline cortico-motor reactivity; contralateral cSP; and three common paired-pulse protocols interleaved in a pseudorandom sequence. Cortico-motor reactivity was assessed by applying 10 single pulses at rest at 120% of RMT, averaging the peak-to-peak amplitude of each MEP (baseline MEP amplitude) and averaging the time from the TMS pulse until the onset of the MEPs (MEP latency). Live EMG was recorded in windows of 5 seconds for cSP measurements. The cSP was assessed with ten single pulses delivered at 120% of RMT during isometric contraction of the FDI at about 25% of their total strength (participants could rest

for few seconds between pulses and had constant visual feedback of their performance). The cSP was measured from the onset of the MEP to the resumption of pre-TMS EMG activity (i.e. relative cSP) (Orth & Rothwell, 2004), and averaged across all 10 trials.

Paired-pulse protocols included SICI, LICI and ICF using standard parameters (Valls-Solé et al., 1992; Kujirai et al., 1993). SICI and ICF consisted of a CP at 80% of RMT, a TP at 120% of RMT and an ISI of 3 and 12 ms, respectively. In LICI, CP and TP were 120% of RMT separated by an ISI of 100ms. Stimulation consisted of 40 individual trials per protocol (for a total of 120 trials), administered in a pseudorandom, interleaved order to reduce blocking effects and with pseudorandomized inter-trial interval (4, 5 or 6 seconds) to minimize expectation and avoid hysteresis. The amplitude of the conditioned MEP for each protocol was averaged across all 40 trials and expressed as a percentage of baseline MEP amplitude. For each TMS measure (except RMT), individual data points > 2.5 SD from the mean were excluded from analysis. For further description of the methodology and physiological implications of the single- and paired-pulse protocols see *Chapter 4 – Section 4.3*. The average values for visits A and B (±SD) as well as the net difference between Visits A and B (Δ_{B-A}) (±SD) and the absolute value of the inter-visit difference ($|\Delta_{B-A}|$) (±SD) for each of the TMS measures are shown in ***Table 7.2***.

Table 7.2. Neurophysiological measures.

	visit A Mean ± SD	visit B Mean ± SD	Δ_{B-A} Mean ± SD	$\lvert\Delta_{B-A}\rvert$ Mean ± SD
RMT (% MSO)				
bi_{AP-PA}	59.7 ± 9.1	59.1 ± 8.8	-0.6 ± 4.2	3.1 ± 2.5
$mono_{AP}$	75.0 ± 7.1	75.0 ± 8.1	0.0 ± 1.4	0.9 ± 1.1
$mono_{PA}$	66.2 ± 4.5	67.0 ± 4.0	0.8 ± 3.5	2.8 ± 2.1
MEP latency (ms)				
bi_{AP-PA}	25.4 ± 1.3	24.8 ± 1.6	-0.6 ± 0.4	0.6 ± 0.4
$mono_{AP}$	24.4 ± 1.8	24.8 ± 1.9	0.4 ± 1.1	1.0 ± 0.6
$mono_{PA}$	23.5 ± 1.7	23.3 ± 2.0	-0.2 ± 0.8	0.6 ± 0.6
MEP amplitude (mV)				
bi_{AP-PA}	1.0 ± 0.6	1.0 ± 0.4	0.0 ± 0.8	0.6 ± 0.5
$mono_{AP}$	1.2 ± 0.4	1.2 ± 0.7	0.0 ± 0.5	0.4 ± 0.3
$mono_{PA}$	1.4 ± 1.0	1.0 ± 0.4	-0.4 ± 0.6	0.6 ± 0.4
cSP (ms)				
bi_{AP-PA}	140.5 ± 27.8	137.3 ± 26.5	-3.3 ± 21.6	16.4 ± 12.8
$mono_{AP}$	129.2 ± 22.2	131.3 ± 37.8	2.2 ± 26.1	17.2 ± 18.4
$mono_{PA}$	122.0 ± 33.8	123.6 ± 30.1	1.6 ± 24.8	18.8 ± 14.9
LICI (%Δ)				
bi_{AP-PA}	-77.9 ± 35.7	-81.4 ± 22.3	-3.4 ± 24.9	14.3 ± 19.9
$mono_{AP}$	-71.4 ± 55.3	-76.3 ± 38.1	-4.9 ± 18.8	9.1 ± 16.9
$mono_{PA}$	-94.9 ± 5.6	-90.00 ± 15.4	4.9 ± 11.3	6.0 ± 10.6
SICI (%Δ)				
bi_{AP-PA}	1.8 ± 85.5	-35.0 ± 75.1	-36.9 ± 80.5	61.7 ± 60.2
$mono_{AP}$	-52.4 ± 38.1	-26.8 ± 120.2	25.6 ± 89.4	41.4 ± 82.2
$mono_{PA}$	-73.6 ± 18.1	-65.4 ± 29.1	8.2 ± 21.0	19.7 ± 9.0
ICF (%Δ)				
bi_{AP-PA}	243.4 ± 177.4	215.6 ± 318.9	-27.7 ± 184.1	127.6 ± 125.6
$mono_{AP}$	101.3 ± 50.4	191.2 ± 206.9	89.9 ± 186.4	126.5 ± 159.7
$mono_{PA}$	0.5 ± 35.3	60.7 ± 85.6	60.2 ± 94.1	87.5 ± 65.5

Abbreviations: Δ_{B-A}, net inter-visit difference; $\lvert\Delta_{B-A}\rvert$, absolute inter-visit difference; *biAP-PA, biphasic anterior-posterior, posterior-anterior; cSP, contralateral cortical silent period; ICC, intraclass correlation coefficient; ICF, intracortical facilitation; LICI, long-interval intracortical inhibition; MEP amp., motor evoked potentials amplitude; MEP lat., motor evoked potentials latency; monoAP, monophasic anterior-posterior; monoPA, monophasic posterior-anterior; MSO, maximal stimulator output; RMT, resting motor threshold; SICI, short-interval intracortical inhibition; %Δ, percent of change from baseline*.

7.2.4 Statistical Analyses

ICC analyses were performed in MATLAB using the Statistics Toolbox (Release 2015b, The MathWorks, Inc., Natick, MA, USA, www.mathworks.com). The software packages Stata (version 13.1, StataCorp LLC, College Station, TX, USA, www.stata.com) and JMP Pro (version 12.0; SAS Institute Inc., Cary, North Carolina, USA, www.jmp.com) were used for the remaining statistical analyses. All analyses were conducted using a two-tailed 95% confidence interval (α=.05).

Calculation of TMS data for each of the three waveforms/current directions ($mono_{PA}$, $mono_{AP}$, $bi_{AP\text{-}PA}$), hereafter referred to as *Waveform*, included: RMT (percent of MSO); unconditioned MEP amplitude (average peak-to-peak amplitude in μV); unconditioned MEP latency (average time in ms from TMS pulse delivery); cSP (duration in ms between the onset of the MEP to the resumption of pre-TMS EMG activity); and three paired-pulse measures, SICI, LICI, and ICF (average peak-to-peak amplitude of conditioned MEPs, expressed as a percent change from baseline; %Δ).

Shapiro–Wilk tests indicated deviations from normality for MEP amplitude, SICI, LICI, and ICF (p's < 0.05), but not RMT, cSP, and MEP latency did not (p's > 0.74). To conform to the assumptions of our parametric statistical tests, baseline MEP amplitude, %Δ SICI, %Δ ICF, and %Δ LICI were transformed as described previously (van Albada & Robinson, 2007).

Data were analyzed in terms of efficacy [1] and reliability [2] using the following approaches:

[1a] Comparison of the magnitude of response to TMS measures across conditions. The response to each TMS measure was entered as a dependent variable into separate mixed-effects analyses of variance (me-ANOVAs) with *Waveform* ($mono_{PA}$, $mono_{AP}$, $bi_{AP\text{-}PA}$) as a between-subjects factor and *Visit* (Visit-A, Visit-B) as a within-subject factor. For MEP latency, Shapiro–Wilk tests indicated the residuals were not normally distributed (p < 0.05), so the ANOVA was

rerun after transforming the data in the manner indicated above. Tukey's honestly significant difference (HSD) tests were used to conduct planned pairwise comparisons between TMS measures obtained with the three *Waveforms*. To control for the effect of potential confounding variables, we added *Gender*, *Inter-Visit Interval* (in days), or *Time Difference* (in minutes) between the starting times of the two visits (one at a time) as a covariate to the above models with the transformed values of TMS measures as dependent variable.

[1b] Assessment of the overall efficacy of paired-pulse TMS protocols in inducing inhibition (SICI and LICI) or facilitation (ICF) of MEPs. Average MEP amplitudes for each paired pulse conditioned were entered into separate mixed-effects ordered logistic regressions (me-OLRs) for each waveform, with *MEP amplitude* as dependent variable, *MEP Type* (conditioned vs. unconditioned) as independent variable and *Visit* as a within-subject factor.

[2] Test-retest reliability was compared across conditions by calculating the ICCs of all TMS measures for each *Waveform* using the ICC formula for absolute agreement (ICC(A,1)) (McGraw & Wong, 1996). In this study we followed the reliability classification which is most commonly adopted in TMS literature, described by Portney and Watkins (Portney & Watkins, 2009). ICC values were interpreted as high (ICC ≥ 0.75), moderate (0.5 ≤ ICC < 0.75), low (0.25 ≤ ICC < 0.5) or very low to none (ICC < 0.25). ICCs are known as the most suitable statistical measurements of reliability (Portney & Watkins, 2009) for continuous quantitative variables, and can be calculated with a confidence interval allowing for statistical comparisons of ICCs between different conditions. To investigate the effect of pulse parameter on test-retest reliability, ICCs were compared across *Waveform* using mixed-effects *F*-statistics (McGraw & Wong, 1996). The effects of *Gender*, *Inter-Visit Interval*, or *Time Difference* on all the ICCs were assessed by including the covariate of interest in the corresponding mixed-effects regression model and recalculating the residual intraclass correlation.

Reliability coefficients, such as the ICCs, can be also used to adjust effect sizes (Baugh, 2002; Wright, 2014). Using this approach, it is possible to predict how the reproducibility of a given

measure might affect a hypothetical effect size, which in turn could be used in a sample size calculation that takes into consideration the reproducibility (or lack thereof) of the measure of interest. To illustrate this point and provide a resource for future studies, adjustments for each measure were made to a hypothetical Cohen's *d* effect size of 0.5, which corresponds to a within-subjects change of half a standard deviation, and is considered a medium effect size (Cohen, 1992). Only positive ICCs values were used to adjust for effect sizes given the nature of the calculations. First, a hypothetical, or *idealized,* Cohen's *d* is converted into an *r* (Cohen, 1988)*:*

$$(1) \qquad r_{IDEALIZED} = d_{IDEALIZED} / (d^2_{IDEALIZED} + 4)^{0.5}$$

Then this *idealized r* is adjusted for unreliability using the ICC (Wright, 2014):

$$(2) \qquad r^2_{ADJUSTED} = r^2_{IDEALIZED} * ICC^{0.5}$$

Finally, the corrected *r* is converted back into an adjusted *d* (Friedman, 1968):

$$(3) \qquad d_{ADJUSTED} = (2 * r_{ADJUSTED}) / (1 - r^2_{ADJUSTED})^{0.5}$$

For between-groups comparisons, the sample sizes in the present study provided 0.80 power to detect a medium effect size (Cohen's d = 0.54).

[3] In addition, we explored the relationship between RMT and the other TMS single- and paired-pulse TMS measures included in the study. Each TMS measure was entered into a separate mixed-effects linear regression with *RMT* as a predictor, *Waveform* as a between-subjects factor, *Visit* as a within-subject factor, and *Waveform*-x-*Visit* interaction. All linear regression analyses were conducted using the transformed values for the TMS measures. For each regression analysis, we checked the bivariate normality between RMT and the other TMS

measure using the Doornik-Hansen test. There was no significant deviation from bivariate normality in any of the regression models (p's > 0.19).

7.3 Results

7.3.1 Comparison of the magnitude of response to single- and paired-pulse measures across Waveforms and visits

The effects of the different TMS measurements in each condition are presented in ***Figure 7.1***. The results of me-ANOVAs with TMS measures as dependent variables and *Waveforms* and visits as predictors are detailed in ***Table 7.3***.

In single-pulse measures, there was a significant overall effect of *Waveform* on RMT (p = 0.001). Pairwise comparisons conducted with Tukey's HSD found that RMT was significantly higher in the $mono_{AP}$ condition than in both the $mono_{PA}$ condition and the bi_{AP-PA} condition (both p's < .05). Furthermore, the $mono_{PA}$ RMT was significantly higher than the bi_{AP-PA} RMT (p < .05). MEP latency was significantly shorter in the $mono_{PA}$ condition than in both the bi_{AP-PA} condition and the $mono_{AP}$ condition (both p's < .05). The cSP duration was significantly shorter with $mono_{PA}$ than with bi_{AP-PA} pulses (p < .05). The cSP was significantly shorter with $mono_{PA}$ than with bi_{AP-PA} (p < 0.05). None of the other pairwise differences in single-pulse TMS measures between the waveforms were significant (p's > 0.05).

Considering the influence of potential confounding variables on single-pulse TMS measures, the only observed relationship was that between MEP latency and *Gender*, which was a significant predictor (p = .002), and controlling for *Gender*, the pairwise differences in MEP latency between $mono_{PA}$ and either $mono_{AP}$ (p < 0.05) or bi_{AP-PA} (p < 0.05), remained significant.

No comparisons of any other single-pulse TMS measure was significantly influenced by *Gender*, *Inter-Visit Interval*, or *Time Difference* (*p* values > .087).

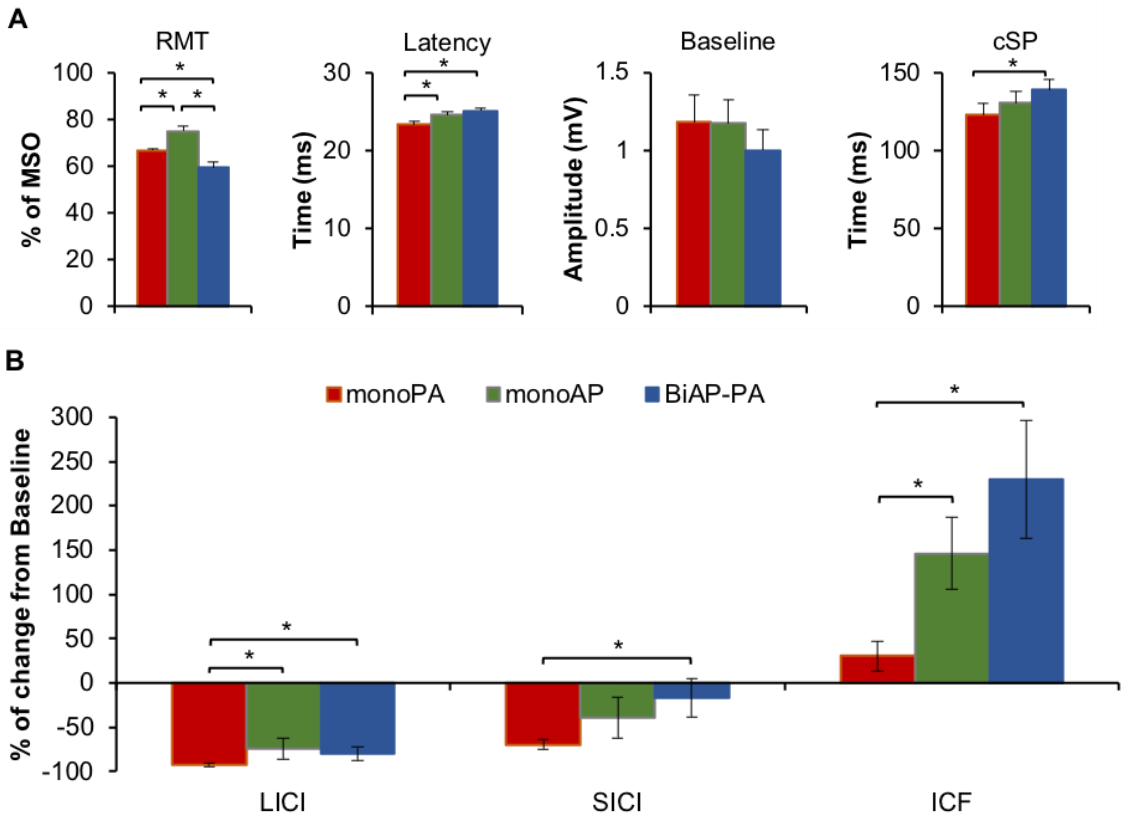

Figure 7.1. Magnitude of the response to single- and paired-pulse TMS.

Results from Tukey's HSD pairwise comparisons (* $p < 0.05$) after me-ANOVAs analysis between waveforms and current directions for each TMS measure. Means (±SE) are shown for each measure. (A) RMT was significantly different between all waveforms and current directions. $Mono_{PA}$ elicited significantly longer MEP latencies than in both the bi_{AP-PA} condition and the $mono_{AP}$ condition and significantly shorter cSP durations than bi_{AP-PA}. (B) In paired-pulse protocols, the $mono_{PA}$ condition yielded to significantly greater inhibition after LICI and shorter facilitation that the other two waveforms. SICI after monoPA led to significantly smaller MEPs than bi_{AP-PA}. *Abbreviations:* bi_{AP-PA}, biphasic anterior-to-posterior—posterior-to-anterior; cSP, cortical silent period; ICF, intracortical facilitation; LICI, long interval intracortical inhibition; $mono_{AP}$, monophasic anterior-to-posterior; $mono_{PA}$, monophasic posterior-to-anterior; MSO, maximal stimulator output; RMT, resting motor threshold; SICI, short interval intracortical inhibition.

For the paired-pulse measures, the percent of change of each conditioned MEP from the unconditioned MEP was calculated and transformed. The transformed values were entered into separate me-ANOVA models, following the same statistical approach as for single-pulse TMS effects described above. The results, showed in ***Table 7.3***, indicated that there was a main effect of *Waveform* for ICF (p = 0.001), but not for SICI or LICI (p's > 0.2). Specifically, Tukey's HSD found that ICF induced a significantly greater facilitation with $bi_{AP\text{-}PA}$ and $mono_{AP}$ than with $mono_{PA}$ pulses ($p's$ < 0.05). Pairwise comparisons conducted with Tukey's HSD found that SICI induced significantly greater inhibition with $mono_{PA}$ than with $bi_{AP\text{-}PA}$ pulses (p < 0.05), but all the waveforms induced similar inhibition with LICI (p's > 0.05). The effects of *Gender*, *Inter-Visit Interval*, or *Time Difference* were not significant in any of the me-ANOVAs on paired-pulse TMS measures (p's > 0.10).

Table 7.3. Results of mixed-effect ANOVAs.

	Model			Waveform			Visit			Waveform x Visit			Tukey's HSD pairwise comparisons
	$F(25,20)$	p	adj-R^2	$F(2,20)$	p	η^2_p	$F(1,20)$	p	η^2_p	$F(2,20)$	p	η^2_p	
RMT (% MSO)	26.06	< 0.001	0.93	**9.28**	**0.001**	**0.48**	0.01	0.981	< 0.01	0.02	0.980	< 0.01	$bi_{AP-PA} < mono_{PA} < mono_{AP}$
MEP latency*	12.33	< 0.001	0.86	2.27	0.129	0.19	0.10	0.751	< 0.01	0.20	0.818	0.02	$mono_{PA} < mono_{AP}$, bi_{AP-PA}
MEP amplitude*	2.25	0.035	0.41	0.07	0.930	0.01	0.06	0.802	< 0.01	0.14	0.869	0.01	*n.s.*
cSP	4.46	< 0.001	0.66	0.66	0.527	0.06	< 0.01	0.989	< 0.01	0.02	0.979	< 0.01	$mono_{PA} < bi_{AP-PA}$
%Δ LICI*	6.76	< 0.001	0.76	0.33	0.724	0.03	0.16	0.696	0.01	0.03	0.972	< 0.01	*n.s.*
%Δ SICI*	5.21	< 0.001	0.70	1.66	0.215	0.14	0.08	0.781	< 0.01	0.48	0.627	0.05	$bi_{AP-PA} < mono_{PA}$, $mono_{AP}$
%Δ ICF*	4.29	< 0.001	0.65	**10.23**	**< 0.001**	**0.51**	0.23	0.634	0.01	1.52	0.242	0.13	$mono_{PA} < mono_{AP}$, bi_{AP-PA}

Variables marked by * were transformed prior to analysis (see text for details). *Abbreviations: adj-R^2, adjusted-R^2; biAP-PA, biphasic anterior-posterior—posterior-anterior; cSP, cortical silent period; ICF, intracortical facilitation; LICI, long-interval intracortical inhibition; MEP, motor evoked potentials; monoAP, monophasic anterior-posterior; monoPA, monophasic posterior-anterior; MSO, maximal stimulator output; n.s., no significant differences; RMT, resting motor threshold; SICI, short-interval intracortical inhibition; %Δ, percentage change from baseline; η2p, partial eta squared.*

7.3.2 Efficacy of paired-pulse protocols across Waveforms and visits

The me-OLRs found that SICI induced an overall significant inhibition of MEPs (z = –5.83, p < 0.001) across all waveforms and visits. Conducting the me-OLR separately for each waveform found significant inhibition with both $mono_{PA}$ (z = –4.81, p < 0.001) and $mono_{AP}$ (z = –3.52, p < 0.001), but not with $bi_{AP\text{-}PA}$ (z = -1.53, p > 0.12). Similarly, LICI induced an overall significant inhibition of MEPs (z = –7.61, p < .001), which was observed across both visits of $bi_{AP\text{-}PA}$ (z = –4.81, p < 0.001), $mono_{AP}$ (z = –3.86, p < 0.001), and $mono_{PA}$ (z = –4.11, p < 0.001). ICF induced a significant overall facilitation of MEPs (z = 5.39, p < 0.001). ICF induced a significant facilitation with both $bi_{AP\text{-}PA}$ (z = 3.69, p < 0.001) and $mono_{AP}$ (z = 4.10, p < 0.001), across both visits, whereas there was no significant facilitation with $mono_{PA}$ (p > 0.31). The effect of *Visit* was not significant in any of the above analyses (p's > 0.05). These results indicate that monoPA and biAP-PA may not be optimal for ICF and SICI, respectively.

7.3.3 Test-retest reliability measures

The ICC values for single- and paired-pulse measures with $mono_{PA}$, $mono_{AP}$, and $bi_{AP\text{-}PA}$ conditions are presented in ***Figure 7.2***. After controlling for *Gender*, *Inter-Visit Interval*, or *Time Difference*, the ICCs for RMT with $bi_{AP\text{-}PA}$ (0.73–0.91) and for LICI with $bi_{AP\text{-}PA}$ (0.65–0.76) varied to some extent, but none of the other ICCs for single- or paired-pulse measures for any of the waveforms changed noticeably, i.e., they did not cross our pre-defined boundaries for interpreting ICC values (see Methods).

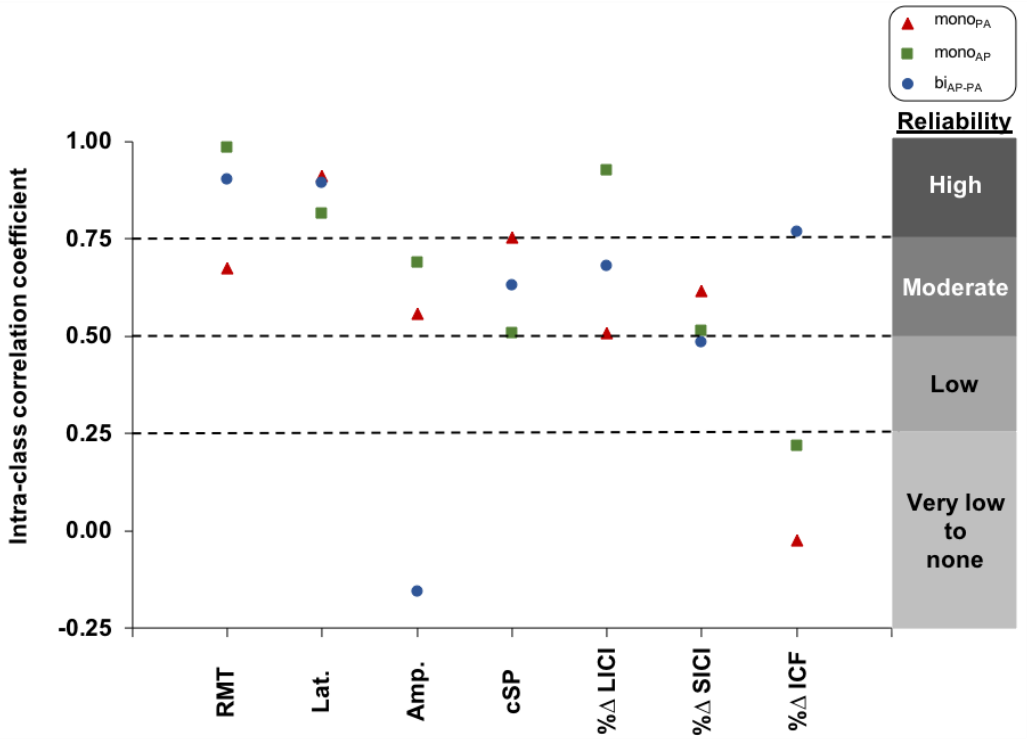

Figure 7.2. Reliability of single- and paired-pulse TMS measures.
Intra-class correlation coefficients (ICCs) for the different TMS protocols performed with different waveforms and current directions in the healthy young adults (ages 18 – 35). *Abbreviations: Amp., baseline MEP amplitude;* bi_{AP-PA}, biphasic anterior-to-posterior—posterior-to-anterior; cSP, cortical silent period; $mono_{AP}$, monophasic anterior-to-posterior; $mono_{PA}$, monophasic posterior-to-anterior; Lat., baseline MEP latency; RMT, resting motor threshold; %Δ LICI, long interval intracortical inhibition percentage of change from baseline; %Δ SICI, short interval intracortical inhibition percentage of change from baseline; %Δ ICF, intracortical facilitation percentage of change from baseline.

Reliability coefficients, pairwise comparisons between the ICC values for TMS measures in the three conditions and hypothetical effect sizes adjusted for these ICCs are detailed in ***Table 7.4***.

Table 7.4. Reliability coefficients and corresponding adjusted effect and sample sizes.

	ICC		ICC comparisons (*p*-values)			Cohen's d	Additional *n* required
	r	*p*	bi_{AP-PA} - $mono_{AP}$	bi_{AP-PA} - $mono_{PA}$	$mono_{AP}$ - $mono_{PA}$		
RMT (% MSO)							
bi_{AP-PA}	**0.90**	**0.001**				0.49	1
$mono_{AP}$	**0.99**	**<.001**	0.983	0.065	0.999	0.50	0
$mono_{PA}$	**0.68**	**0.016**				0.45	7
MEP latency (ms)							
bi_{AP-PA}	**0.89**	**0.018**				0.49	1
$mono_{AP}$	**0.82**	**0.004**	0.384	0.385	0.133	0.47	4
$mono_{PA}$	**0.91**	**<.001**				0.49	1
MEP amplitude (mV)							
bi_{AP-PA}	-0.16	0.621				-	-
$mono_{AP}$	**0.69**	**0.039**	**0.021**	**0.009**	0.313	0.45	7
$mono_{PA}$	**0.56**	**0.030**				0.43	11
cSP (ms)							
bi_{AP-PA}	**0.71**	**0.028**				0.46	6
$mono_{AP}$	**0.68**	**0.041**	0.557	0.475	0.408	0.45	7
$mono_{PA}$	**0.72**	**0.012**				0.46	6
LICI (%∆)							
bi_{AP-PA}	**0.68**	**0.038**				0.45	7
$mono_{AP}$	**0.93**	**<.001**	**0.030**	0.776	**0.007**	0.49	1
$mono_{PA}$	0.51	0.051				0.42	13
SICI (%∆)							
bi_{AP-PA}	0.48	0.088				0.41	15
$mono_{AP}$	0.51	0.095	0.530	0.290	0.633	0.42	13
$mono_{PA}$	**0.62**	**0.021**				0.44	9
ICF (%∆)							
bi_{AP-PA}	**0.77**	**0.015**				0.47	4
$mono_{AP}$	0.22	0.276	**0.040**	**0.014**	0.256	0.34	36
$mono_{PA}$	-0.02	0.535				-	-

Abbreviations: biAP-PA, biphasic anterior-posterior, posterior-anterior; cSP, contralateral cortical silent period; ICC, intraclass correlation coefficient; ICF, intracortical facilitation; LICI, long-interval intracortical inhibition; MEP, motor evoked potentials; monoAP, monophasic anterior-posterior; monoPA, monophasic posterior-anterior; MSO, maximal stimulator output; RMT, resting motor threshold; SICI, short-interval intracortical inhibition; %∆, percent of change from baseline. Significant values are shown in bold type.

Baseline MEP amplitude was significantly more reliable when obtained with monophasic pulses, whether $mono_{PA}$ or $mono_{AP}$, than with $bi_{AP\text{-}PA}$ pulses (both *p*'s < 0.022). LICI was significantly more reliable when obtained with $mono_{AP}$ pulses than with either $mono_{PA}$ or $bi_{AP\text{-}PA}$ pulses (both *p*'s < 0.031), whereas ICF was significantly more reliable when obtained with $bi_{AP\text{-}PA}$ pulses than both monophasic pulses (both *p*'s < 0.041). The ICCs for other TMS measures were not significantly different between the three conditions (all *p*'s > 0.064). Notably, those protocols that were found to have moderate to high reliability, also had a Cohen's d close to the a *priori* idealized Cohen's d effect size of 0.5 and very low additional *n* were required to detect the attenuated effects.

7.3.4 Relationship between RMT and other TMS measures

The exploratory mixed-effects linear regressions assessing the relationship between *RMT* and the transformed values of single- and paired-pulse TMS measures across the two visits found a significative negative association between RMT and baseline MEP amplitude (*p* = 0.04). None of the associations between RMT and other TMS measures were significant (*p*'s > 0.23).

7.4 Discussion

In the present experiment, we investigated the influence of specific TMS physical or technical factors, i.e. pulse waveform and current direction ($mono_{PA}$, $mono_{AP}$, and $bi_{AP\text{-}PA}$), on the efficacy and test-retest reliability of common single- and paired-pulse TMS measures in young healthy adults. Pulse waveform/current direction was observed to exert the greatest influence on RMT, MEP latency, cSP and the two of the three paired-pulse protocols that were explored (SICI and ICF). $Mono_{AP}$ led to higher RMTs, followed by $mono_{PA}$ and $bi_{AP\text{-}PA}$ pulses. $Mono_{PA}$ pulses

resulted in the shortest MEP latency, shortest cSP duration and the greatest LICI and SICI, but the greatest ICF was achieved with waveforms with an AP component (either $mono_{AP}$ or bi_{AP-PA}). There were also significant effects of waveform/current direction on test-retest reliability of baseline MEP amplitude, LICI, and ICF. $Mono_{PA}$ pulses resulted in a more reliable baseline MEP amplitude, but less reliable ICF than bi_{AP-PA} pulses that showed the greatest reliability. In contrast, LICI was more reliable with $mono_{AP}$ than with $mono_{PA}$ pulses.

The results of the present study can be interpreted using a framework put forth by Di Lazzaro and Rothwell (Di Lazzaro & Rothwell, 2014) following a series of experiments performed on patients with epidural electrodes implanted at the cervical spinal cord level (Di Lazzaro et al., 2001, 2003; Di Lazzaro et al., 2011). As described in more depth in *Chapter 2 – Section 2.3,* the authors proposed that different waveforms and current directions interact with stimulation intensity to evoke distinct patterns of D- and I-waves by selective recruitment of particular neural components of cortical layers. For example, $mono_{PA}$ pulses at threshold intensities elicit an early I-wave (the I1-wave), which is thought to reflect indirect monosynaptic activation of layer V PTNs through excitatory interneurons in cortical layers II and III. As the intensity of $mono_{PA}$ pulses increases, descending volleys begin to include later I-waves, which are thought to reflect polysynaptic chains of interneurons in the same layers II and III acting on layer V PTNs. In contrast, $mono_{AP}$ pulses tend to evoke late I-waves that are more dispersed and have longer latencies. These late I-waves are thought to reflect the activation of horizontal cortico-cortical connections in layers II-III that originate from surrounding regions, probably premotor cortex or the thalamus, but perhaps other brain structures. As such, $mono_{AP}$ currents typically result in higher motor thresholds than $mono_{PA}$ currents. Biphasic pulse waveforms elicit a more complex pattern of D- and I-waves and the role of current direction (AP-PA versus PA-AP) has not been well elucidated. When using biphasic pulses at threshold intensities, the second phase of the waveform seems to be more relevant to stimulating the cortex. Further, it has been reported using PA-AP currents that at threshold intensities, the AP and PA phases may be partially antagonistic

with respect to polarization of the membrane and opening/closing of Na^+ channels necessary to generate an action potential (Rothwell, 2017). As the intensity of biphasic pulses increases, however, the first phase begins depolarizing sensitive neural elements, adding to the overall response (Di Lazzaro et al., 2003; Barker, 2017). One consequence of this complex relationship is that biphasic pulses at suprathreshold intensities tend to be less direction-dependent and can elicit a combination of D- and I-waves (Di Lazzaro et al., 2001, 2003; Di Lazzaro et al., 2011).

The present study, to our knowledge, is the first to assess the effects of induced current direction and pulse waveform on both the efficacy and test-retest reliability of different TMS measures including paired-pulse TMS measures (SICI, LICI, and ICF). These paired-pulse measures allow for exploring inhibitory vs. excitatory effects of specific populations of intracortical neural components on the final output of PTNs, where inhibitory processes and long-interval excitatory effects are mainly mediated by GABAergic and glutamatergic activity, respectively (Sohn et al., 2002; Ziemann et al., 2002; Di Lazzaro et al., 2006; Lang et al., 2006; Ziemann, 2013).

In *Methodology* (*Chapter 4 – Section 4.3*) of the present thesis, we have described in depth the physiological processes that take place during TMS-elicited inhibition or facilitation in the motor system. As a brief reminder of those processes, epidural recordings of paired-pulse TMS protocols have only been conducted with $mono_{PA}$ stimulation, it is unknown how other pulse waveforms or current directions would influence the effects of paired-pulse paradigms on the descending volleys. With $mono_{PA}$ pulses, both SICI and LICI suppress the I2 and later waves, but not the D- or I1-waves (Nakamura et al., 1996; Di Lazzaro, Restuccia, et al., 1998; Di Lazzaro et al., 2002; Ni et al., 2011). While SICI is thought to be mediated by $GABA_A$ receptor-dependent pathways (Di Lazzaro et al., 2000, 2006; Teo et al., 2009; Ziemann, 2013), the longer-interval inhibition caused by LICI is thought to reflect the inhibitory post-synaptic potential mediated by $GABA_B$ receptors (Werhahn et al., 1999; Ziemann, 2013). Unlike SICI and LICI, however, ICF does not significantly change the amplitude or number of corticospinal waves (Di Lazzaro et al.,

2006; Ni et al., 2011), indicating that the ICF-induced increase in MEP amplitude might reflect the recruitment of neural circuits unrelated to those involved in the generation of I-waves elicited by $mono_{PA}$. Such recruitment can result in more dispersed activity that is not reflected in epidural recordings (Di Lazzaro & Rothwell, 2014). As mentioned in *Methodology* (*Chapter 4 – Section 4.3*), although it is most likely that the origin of ICF is cortical (Cash et al., 2017), a complementary theory for the neural source of ICF has been evaluated recently (Wiegel et al., 2018), suggesting that the subthreshold conditioning pulse of ICF is able to trigger subcortical and spinal processes that may contribute to the facilitation of MEPs.

7.4.1 Effects of pulse waveform/current direction on the response to TMS measures

We found significant differences in RMT, MEP latency, cSP, SICI, LICI, and ICF between the three conditions. The only TMS measure where the statistical analysis showed no influence of the explored waveforms/current directions was baseline MEP amplitude. $Mono_{AP}$ pulses yielded the highest RMT followed by $mono_{PA}$ and bi_{AP-PA}. These findings are consistent with the results of previous studies that compared $mono_{PA}$ and biphasic waveforms (Niehaus et al., 2000; Kammer et al., 2001; Sommer et al., 2006; Delvendahl, Gattinger, et al., 2014; Stephani et al., 2016) as well as $mono_{PA}$ and $mono_{AP}$ current directions (Sakai et al., 1997; Orth & Rothwell, 2004; Delvendahl, Lindemann, et al., 2014). Together, these results support a model of current-cortex interactions whereby the corticospinal pathway is most efficiently stimulated with bi_{AP-PA} pulse waveforms followed by $mono_{PA}$ and $mono_{AP}$ current directions induced orthogonally to the central sulcus.

In contrast with our finding that RMT with biphasic pulses was the lowest among the three conditions, Orth and Rothwell (Orth & Rothwell, 2004) found the RMT to be higher with biphasic pulses than with either $mono_{AP}$ or $mono_{PA}$ pulses. These different results can be due to two factors:

(1) The induced current direction in the Orth and Rothwell study (PA-AP) was opposite to that in the present study (AP-PA) and, thus, could have altered the interactions of the phases. While at threshold intensities the PA component of the biphasic pulse is likely the primary contributor to the MEP, it is possible that the AP component may have an agonistic effect if it is first and an antagonistic effect if it is second. Future studies could resolve this by directly comparing the TMS measures obtained with $bi_{AP\text{-}PA}$ and $bi_{PA\text{-}AP}$ pulses using the same stimulator; (2) We used a MagVenture MagPro device, whereas Orth and Rothwell used a Magstim 200 stimulator (Magstim Co. Whitland, Dyfed, UK). As previously mentioned in *State of the Art* (*Chapter 2 – Section 2.2*), Kammer and colleagues (Kammer et al., 2001) compared devices from Magstim and MagVenture companies and obtained lower RMTs with monophasic than with biphasic pulses when using Magstim, whereas MagVenture led to lower biphasic thresholds regardless of current direction. These results suggest that different devices may have different total stimulation strengths depending on the waveform.

Our results are in agreement with prior studies that found the MEP latencies to be shorter with $mono_{PA}$ than with $mono_{AP}$ pulses (Mills et al., 1992; Takahashi et al., 2005; Sommer et al., 2006; Ni et al., 2011; Delvendahl, Gattinger, et al., 2014; Delvendahl, Lindemann, et al., 2014; D'Ostilio et al., 2016), probably because different current directions activate different neural components with distinct latencies within the corticospinal pathway (Di Lazzaro & Rothwell, 2014). Moreover, our results show a difference between $mono_{AP}$ and $mono_{PA}$ latencies of about 1.2 ms. This difference is in line with the results from Di Lazzaro (Di Lazzaro et al., 2001, 2003; Di Lazzaro et al., 2011) and probably reflects that $mono_{AP}$ pulses elicit later and more dispersed I-waves.

In contrast, discrepant results have been reported when comparing the latency of MEPs obtained with monophasic and biphasic pulses. While some studies found longer latencies with $mono_{AP}$ than with biphasic pulses (Sommer et al., 2006; Delvendahl, Gattinger, et al., 2014), other studies found no signifcant difference between the three waveform/current directions (Niehaus et al., 2000). Following Di Lazzaro and Rothwell's theoretical model (Di Lazzaro & Rothwell, 2014),

we would expect that biphasic pulses elicit MEPs with shorter latencies, since at high-enough intensities, a biphasic pulse evokes a D-wave reflecting the direct activation of the PTNs. Our results, however, showed that $bi_{AP\text{-}PA}$ MEP latencies were longer than $mono_{PA}$, and comparable to $mono_{AP}$, latencies. Although these results may appear contradictory, they could be due to several factors: (1) Our data show a difference in MEP latency between $mono_{PA}$ and $bi_{AP\text{-}PA}$ of about 1.7 ms. This may be due to the fact that the neural components activated by $bi_{AP\text{-}PA}$ had a longer latency, similar to the ones activated by $mono_{AP}$ (in our study the difference between $mono_{PA}$ and $mono_{AP}$ latencies was 1.2ms). (2) It is possible that the intensity of the biphasic pulse was not high enough to reach layer V of the motor cortex or to overcome the PTNs' firing threshold and therefore, the activation of the PTN's was indirect. Previous studies have shown that biphasic pulses at 120% of RMT might not activate the PTNs directly and hence do not elicit D-waves (Di Lazzaro et al., 2001) but elicit a complex group of I-waves with longer latencies. (3) At threshold levels, the PA phase as second component of the $bi_{AP\text{-}PA}$ pulse has a greater importance, whereas the AP phase gains more relevance as the stimulation intensity is increased to suprathreshold levels. Considering that MEP latency was 1.7 ms longer with $bi_{AP\text{-}PA}$ than with $mono_{PA}$ pulses, it is possible that in our study, the AP component played a more relevant role in the activation of the motor cortex. Therefore, the PA and AP components could have worked against each other in activating the inhibitory and excitatory interneuron networks, hence leading to longer latecies. We hasten to add that this hypothesis is based on insufficient evidence in the literature and needs to be investigated in future studies, for example by comparing the latencies of MEPs elicited with the different waveforms and current directions at different intensities in a input-ouput curve (see *Chapter 4 – Section 4.2* for the description and diagram of an input-ouput curve). Lastly, when controlling for potential confounding factors, we found that gender significantly influenced MEP latencies. This relationship has been described in previous studies and is considered to be due to a difference in limbs length between genders (Livingston, Goodkin, & Ingersoll, 2010).

Contradictory results have also been reported regarding the effects of waveform and current direction on MEP amplitude (Mills et al., 1992; Takahashi et al., 2005; Sommer et al., 2006; Ni et al., 2011; Delvendahl, Gattinger, et al., 2014; Delvendahl, Lindemann, et al., 2014; D'Ostilio et al., 2016). As previously argued in *State of the Art* (*Chapter 2 – Section 2.5*), an additional source of variability is differences in methodology among previous studies: while some studies used a fixed portion of MSO to elicit MEPs , other studies used a specific percentage of RMT to assess the effect of waveforms/current directions on MEP amplitude (Delvendahl, Gattinger, et al., 2014; Delvendahl, Lindemann, et al., 2014). Our finding that MEP amplitudes elicited at 120% of RMT were not significantly different between the three conditions is consistent with the results of previous studies that used similar TMS parameters (Delvendahl, Gattinger, et al., 2014; Delvendahl, Lindemann, et al., 2014).

With the FDI slightly contracted, $bi_{AP\text{-}PA}$ yielded longer cSP durations than $mono_{PA}$, with $mono_{AP}$ in between. These results are generally consistent with the findings of previous cSP studies (Orth & Rothwell, 2004; Sommer et al., 2006). Moreover, the similarity in cSP duration between $mono_{PA}$ and $mono_{AP}$ pulses reflected in our data is consistent with the results reported by Sommer and colleagues (Sommer et al., 2006), but contrasts with those reported by Orth and Rothwell (Orth & Rothwell, 2004), who observed shorter cSP durations with $mono_{PA}$ pulses than with either $mono_{AP}$ or $bi_{PA\text{-}AP}$ pulses. These different results can be due to several factors: First, Orth and Rothwell used a Magstim 200 stimulator for monophasic pulses and a Magstim Super Rapid stimulator for biphasic pulses (Magstim Co., Whitland, Dyfed, UK), whereas both we and Sommer and colleagues used a MagPro X100 stimulator for all conditions. As mentioned above and in *State of the Art*, it has been shown that the maximal intensities of the induced magnetic field vary across stimulators (Kammer et al., 2001) and waveforms, which may influence the cSP duration. Second, Orth and Rothwell used 150% of active motor threshold as the stimulation intensity, whereas both the present study and that from Sommer and colleagues set the stimulation intensity based on RMT. Therefore, our results are in agreement with those of Sommer

et al.'s study, in which the technical TMS pulse parameters were mostly similar to ours but differ to some extent (cSP was not significantly different between $mono_{AP}$ and $mono_{PA}$) from those studies in which the cSP was performed with a different stimulator and with different stimulation parameters.

In sum, RMT was lowest with $bi_{AP\text{-}PA}$ and highest with $mono_{AP}$, latencies were shorter with $mono_{PA}$, whereas MEP amplitudes were comparable in the three conditions. These findings indicate that different pulse waveforms/current directions may recruit different subgroups of interneurons at different intensities (Di Lazzaro & Rothwell, 2014). For example, $bi_{AP\text{-}PA}$ pulses seem to be more efficient at threshold levels but elicit non-significantly smaller MEPs at higher intensities.

Paired-pulse protocols have been conventionally performed with $mono_{PA}$ pulses, probably due to historical reasons and technical availability when they were first described. Our results show that monophasic pulses resulted in stronger short intracortical inhibition (SICI), but weaker facilitation (ICF), when measured with $mono_{PA}$. Interestingly, significant facilitation (compared to baseline) was only achieved in the two conditions that included an AP component (i.e., $mono_{AP}$ and $bi_{AP\text{-}PA}$).

Although the physiological mechanisms responsible for the results of measures of intracortical balance of inhibition and facilitation (i.e., cSP and paired pulse TMS) cannot be directly inferred from the present study, some hypotheses can be formulated. The results suggest that $mono_{PA}$ waveforms may be more efficient in targeting short-interval inhibitory cortical mechanisms. Based on invasive epidural recordings showing a reduction of I2- and late I-wave amplitudes from LICI and SICI performed with $mono_{PA}$ pulses (Nakamura et al., 1996; Di Lazzaro, Restuccia, et al., 1998; Di Lazzaro et al., 2002, 2011), the present results are consistent with the hypothesis that $mono_{PA}$ pulses activate interneuron networks in layers II and III of the motor cortex that inhibit layer V PTNs. However, no effect on the amplitude of D- or I-waves was observed when performing ICF with $mono_{PA}$. In our study, performing ICF with $mono_{PA}$ pulses induced a

small facilitation that was not significantly different from baseline. On the other hand, pulse waveforms with an AP component ($mono_{AP}$ and bi_{AP-PA}) led to significant facilitation. So far, the influence of AP currents on D- and I-waves during facilitatory protocols has only been studied in a single subject (Di Lazzaro et al., 2006) showing the influence of ICF on late I-waves (I4- and I5-waves). Additional insights to the relationship between AP currents and ICF may come from the results of cSP. Even though cSP is an inhibitory protocol conducted with a single suprathreshold pulse, it is dependent on voluntary muscle contraction, which may reflect the engagement of additional cortical (i.e., premotor or supplementary motor areas) and/or subcortical structures. Similar to the results with ICF, cSP seems to be longer with pulses that include an AP component. If AP-oriented currents target inputs to primary motor cortex from surrounding cortices or other brain structures, the present results support the hypothesis that these cortico-cortical connections may subserve the processes that underlie both cSP and ICF. Although, this hypothesis needs to be confirmed in future studies with epidural recordings.

Finally, we examined the associations between RMT and the other TMS measures, and found the baseline MEP amplitude to be the only TMS measure that was related to RMT. The negative association between these two measures was also observed in the other experiment of TMS reliability of the present work (see *Chapter 8* for further information).

7.4.2 Effects of pulse waveform/current direction on the reliability of TMS measures

Based on Portney et al. (Portney & Watkins, 2009) categorization of reproducibility for neurophysiological assessments as well as in TMS literature, moderate to high reliability was observed in all measures across waveforms/current directions with the exception of baseline MEP amplitude with biphasic pulses and ICF with monophasic pulses regardless of current direction. Waveform/current direction significantly influenced the test-retest reliability of baseline MEP

amplitude, LICI, and ICF. The bi_{AP-PA} pulses resulted in less-reliable baseline MEP amplitude, but more-reliable ICF, than $mono_{PA}$ pulses. In contrast, LICI was more reliable when obtained with $mono_{AP}$ than with $mono_{PA}$ pulses.

The intensities of most TMS protocols are determined based on RMT. In *State of the Art* we reviewed previous publications that studied the reliability of TMS and patterns can be observed in ***Figure 2.8*** (*Chapter 2 – Section 2.5*) of the present thesis. That review pointed out that, not surprisingly, RMT (Carroll et al., 2001; Kimiskidis et al., 2004; Livingston & Ingersoll, 2008; Fleming et al., 2012; Ngomo et al., 2012; Liu & Au-Yeung, 2014; Schambra et al., 2015; Hermsen et al., 2016) is the most studied variable in TMS literature and is also the most reliable TMS measure, followed by MEP latency (Livingston & Ingersoll, 2008; Bastani & Jaberzadeh, 2012; Hoonhorst et al., 2014). Our reliability results on RMT and MEP latency are in line with those reported previously.

From the brief review, it is also worth noting that the results of previous studies have reported a wide range of test-retest reliability of MEP amplitude (Carroll et al., 2001; Kamen, 2004; Kimiskidis et al., 2004; McDonnell et al., 2004; Christie et al., 2007; Livingston & Ingersoll, 2008; Bastani & Jaberzadeh, 2012; Fleming et al., 2012; Ngomo et al., 2012; Liu & Au-Yeung, 2014; Sankarasubramanian et al., 2015; Schambra et al., 2015; Hermsen et al., 2016). This variability across studies is of great relevance given that most TMS studies report the mean amplitude of a number of MEPs as their baseline and special consideration should be put on factors that may play a role on the heterogeneity of the results. Controlling the pulse waveform/current direction, optimally monophasic (in either PA or AP direction), may improve the reliability of the MEP amplitude.

The reliability of cSP (Liu & Au-Yeung, 2014; Hermsen et al., 2016) and paired-pulse TMS measures (Fleming et al., 2012; Ngomo et al., 2012; Schambra et al., 2015; Hermsen et al., 2016) have not been adequately studied and ***Figure 2.8*** of *State of the Art* shows that the evidence is very scarce thus far. Furthermore, both cSP and paired-pulse TMS measures have only been

studied with $mono_{PA}$ pulses. Our results on cSP and SICI show moderate reliability regardless of the waveform/current direction. In contrast, LICI that has been very poorly studied (Schambra et al., 2015), shows greater reliability with monoAP. Particular attention should be paid to ICF given that its reliability in the present work was excellent when obtained with biphasic, but not with monophasic, pulses. This may partially explain the low reliability of ICF that has been previously reported since only $mono_{PA}$ pulse have been used.

Furthermore, reliability coefficients such as ICC's can be used to adjust effect sizes to account for the fact that those calculations are made under the implicit assumption of perfect test-retest reliability (Baugh, 2002; Wright, 2014). In other words, detecting a significant change of any given size in a longitudinal design is more difficult for an unreliable measure than for a reliable one. In turn, an adjusted effect size can be used to provide a more realistic estimate of the sample size required to observe the desired effect given the reproducibility of the measure being tested. ***Table 7.4*** shows adjustments to a hypothetical Cohen's *d* of 0.5, which corresponds to a change of half a standard deviation, for each of the measures in the current analysis. ***Table 7.4*** also shows the sample sizes required to detect the attenuated effects. The results of the present analyses can thus be used to more accurately plan the parameters of single- and paired-pulse TMS-based neurophysiological measures more adequately in relation to the specific outcome of future studies.

7.5 Conclusions

The results presented above show that pulse waveform and current direction influence the efficacy and the reliability of single- and paired-pulse TMS measures and, therefore, should be taken into consideration in assessing TMS measures and also when considering those measures for future studies. In addition, sample sizes have been proposed to guide future investigators in detecting attenuated effects with the analyzed TMS protocols.

These pulse parameters are of special relevance for measuring the RMT or the baseline MEP amplitude, which are the most important and widely used TMS measures. Pulse waveforms and current directions that were not previously studied with paired-pulse measures ($mono_{AP}$ and $bi_{AP\text{-}PA}$) induced significant inhibition (SICI and LICI) or facilitation (ICF) of MEPs. Monophasic pulses induced greater and more reliable inhibition, whereas biphasic pulses induced greater and more reliable facilitation in ICF. Thus, biphasic pulses may be better suited for exploring the effects of TMS when more than one cortical area or brain structure are involved, as in the case of cSP or ICF. These findings can help future studies choose the parameters of the TMS pulse so as to maximize the efficacy and reliability of single- and paired-pulse TMS measures and thus optimize their utility as potential neurophysiologic biomarkers in health and disease.

8 Reliability of single-pulse, paired-pulse, and intermittent Theta-Burst TMS measures in Healthy Aging, Type-2 Diabetes, and Alzheimer's Disease

8.1 Introduction

Thus far we have investigated controllable technical aspects and their influence on the reliability of TMS. However, these factors are not the only ones that have an impact on the variability of the responses to TMS. Changes in brain's physiology related to healthy and pathological aging more than likely change the effects and the reliability of common TMS protocols. As we have already mentioned in *State of the Art* (*Chapter 2 – Section 2.4*), several studies have proven that plasticity mechanisms are among the most important changes with advancing age, moreover those plasticity processes have also been shown to be altered in diseases like AD and T2DM (Babiloni et al., 2016; Gispen & Biessels, 2000; Pascual-Leone et al., 2011). In the successive experiments we will examine how elements related to changes in brain physiology with advancing age or neuropathophysiological processes of common age-related diseases with impaired cognition or glucose metabolism may change the effects and reliability of common TMS protocols.

In order to investigate this, we have focused our analyses on the protocol that is most commonly used for evaluating plasticity with TMS, i.e. TBS. Over the past decade, the so called TBS, an ultra-high frequency patterned rTMS (see *Chapter 4 – Section 4.4* for further information), has emerged as a potential means to generate greater and longer-lasting neuromodulatory effects with a shorter duration of stimulation (Huang et al., 2004). Both the continuous and intermittent forms of TBS protocols have been used to identify age-related changes in the mechanisms of plasticity across the lifespan in healthy individuals (Freitas et al., 2011) and reveal altered

neuroplastic mechanisms in several diseases. Of particular importance for the present work, these altered mechanisms have been observed in T2DM (Fried et al., 2017), and AD (Koch et al., 2012).

While the effects of TBS on healthy and patient population have been tested, the reliability of TBS and possible influencing factors has been insufficiently studied (see *Chapter 2 – Section 2.5 Reliability* for further information). For example, it has been demonstrated that activation of the target muscle prior to (Goldsworthy, Muller-Dahlhaus, Ridding, & Ziemann, 2014), during (Huang et al., 2008), or immediately after TBS (Iezzi et al., 2008) can influence its effects on motor cortex excitability. In addition, carriers of the *BDNF*-Met allele may show altered response to neuromodulation paradigms including TBS (Cheeran et al., 2008; Lee et al., 2013; Di Lazzaro et al., 2015). Despite increased attention, only four studies (Hinder et al., 2014; Vernet et al., 2013; Vallence et al., 2015; Schilberg et al., 2017) have directly assessed the reproducibility of TBS after-effects, and these largely focused on young healthy individuals. Similarly, while there have been more studies investigating the reproducibility of single- and paired-pulse TMS measures, only two (Kimiskidis et al., 2004; Fleming et al., 2012) included subjects over the age of 50, and only one (Christie et al., 2007) exclusively recruited individuals over 65 years.

Aging and age-related pathologies most probably have not only an impact on the outcome of plasticity measure but also on its reliability. The growth in popularity of TMS techniques and their fast expansion throughout the clinical practice as diagnostic and prognostic tools has led to an increased focus on the sources of inter- and intra-individual variability. As interest grows in using TMS and TBS to assess the intracortical and corticospinal excitability and the efficacy of neuroplastic mechanisms in older clinical populations (Freitas et al., 2011; Di Lorenzo et al., 2016; Fried et al., 2017), it is critical to understand the reliability of these techniques in the populations of interest. Moreover, identifying the factors that impact the reliability of TBS and measuring that impact will help the scientific community to elucidate the best clinical and therapeutical use of this technique in two of the most prevalent age-related diseases nowadays, T2DM and AD.

The present study aims to fill this void through a direct assessment of the reproducibility of iTBS after-effects and other common single- and paired- pulse TMS-based neurophysiological measurements in older adults, including those with impaired cognition or glucose metabolism. The results from this study will serve as a guidepost for understanding how biomarkers of cortical reactivity and plasticity change with age or are affected by common diseases such as T2DM and AD.

8.2 Methods

8.2.1 Participants

Retrospective data was obtained from 36 adults of mean age 62.9 years (age range, 50 – 79 years, 17 females), who had participated in a research study between May 2012 and May 2015. The participants were drawn from different populations: nine participants (4 males, mean ± SD age: 67.7 ± 6.9 years) had a probable diagnosis of mild-to-moderate AD (McKhann et al., 2011) with a clinical dementia rating (CDR) = 1.0 and a MMSE between 18-23; 15 participants (9 males, mean ± SD age: 63.4 ± 7.3 years) had a clinical diagnosis of T2DM but were otherwise cognitively intact (MMSE ≥ 27), and the remaining 12 healthy controls (6 males, mean ± SD age: 58.6 ± 9.1 years) were both cognitively intact (MMSE ≥ 27) and non-diabetic (hemoglobin A1c < 6.2%). AD participants consisted of individuals who were randomized to a Sham-control group for a proof-of-principle study on the combined impact of daily rTMS and cognitive training (Brem et al., under review). T2DM and control participants were recruited for a study on cortical plasticity in T2DM (Fried et al., 2017). None of the participants had any unstable medical condition or comorbidity. Saliva was obtained from 24 participants (10 controls, 10 T2DM, 4 AD) to assess *BDNF* and apolipoprotein-E (*APOE*) polymorphisms. All participants underwent anatomical MRI

scan, structured neurological exam, medical history review, formal neuropsychological testing, and two identical TMS visits. Median time between TMS visits was 14 days (range: 2 – 344 days). Average (± SD) start time for the two TMS visits was 10:57 (± 1:07) for Visit-A and 10:37 (± 0:55) for Visit-B. ***Table 8.1*** details participants' characteristics. During both sessions, all participants underwent two TMS safety forms to screen for possible contraindications (see appendix C) and side effects (see appendix E). These TMS safety screening forms were based on the safety guidelines for TMS by The Safety of TMS Consensus Group (Rossi et al., 2009), for more information read *Chapter 2 – Section 2.6* of the present thesis. Prior to the first visit, a T1-weighted anatomical magnetic resonance imaging scan was obtained in all participants and used for neuronavigation. Scans were completed on a 3T scanner (GE Healthcare, Ltd., UK) using a 3D spoiled gradient echo sequence: 162 axial-oriented slices for whole-brain coverage; 240-mm isotropic field-of-view; 0.937-mm x 0.937-mm x 1-mm native resolution; flip angle = 15°; TE/TR ≥ 2.9/6.9 ms; duration ≥ 432 s. Blood glucose levels were assessed in all T2DM subjects at the beginning of each TMS visit for the purpose of establishing that glucose levels were within a normative range defined *a priori* as 80-200 mg/dL.

8.2.2 Electromyography

Surface EMG activity was recorded from the dominant hand's FDI using a an integrated nTMS-EMG Nexstim system (eXimia NBS 4, Nexstim Plc, Finland). As mentioned in *Methodology* (*Chapter 6 – Section 6.1*), electrodes were placed over the FDI in a belly-tendon montage. Live EMG was monitored throughout the protocol to provide feedback of muscle relaxation. MEP peak-to-peak amplitudes (mV) of the non-rectified signal were recorded for individual traces. Participants were comfortably seated with their arms resting in a natural angle, monitored for drowsiness and asked to keep their eyes open throughout the experiment.

Table 8.1. Participant characteristics.

Group	Gender	*BDNF*	*APOE*	Age (y)	Education (y)	MMSE (#/30)	Difference between visits		
							Days	Time (h) (A)	Time (h) (B)
Control	F	Val/Val	e3/e4	56	12	30	13	10:50	9:11
Control	M	Val/Val	e3/e3	53	16	29	7	10:24	9:56
Control	F			51	14	29	15	13:11	10:43
Control	F	Val/Val	e3/e3	50	17	29	24	9:29	10:44
Control	F	Val/Val	e3/e3	74	17	30	3	9:27	9:37
Control	F	Val/Val	e2/e3	56	16	30	14	11:10	11:10
Control	M	Val/Val	e3/e3	51	16	29	8	10:17	9:58
Control	M	Val/Met	e3/e4	61	20	30	49	13:19	12:21
Control	M	Val/Val	e3/e3	77	18	30	48	9:53	9:46
Control	F			50	14	30	13	9:53	9:44
Control	M	Val/Val	e2/e3	60	12	28	3	9:43	9:17
Control	M	Val/Val	e3/e4	64	21	30	2	10:09	10:22
T2DM	M	Val/Val	e2/e3	59	12	28	6	10:13	10:38
T2DM	M	Val/Val	e3/e3	77	18	30	7	12:24	11:28
T2DM	M	Val/Val	e3/e3	64	20	28	2	11:41	11:12
T2DM	M	Val/Val	e3/e4	69	19	30	6	11:25	10:44
T2DM	M			62	14	30	14	8:57	8:18
T2DM	F			50	16	30	7	11:27	11:02
T2DM	F	Val/Val	e2/e3	71	18	30	28	12:58	10:17
T2DM	F	Val/Met	e3/e3	67	14	28	4	10:53	10:00
T2DM	M	Val/Met	e3/e3	53	16	28	3	11:06	10:45
T2DM	M			54	12	27	7	10:17	11:29
T2DM	M	Val/Val	e3/e3	67	14	30	12	9:58	9:31
T2DM	M			67	16	30	12	9:53	9:11
T2DM	F			66	16	30	18	10:41	8:39
AD	M			69	16	22	49	10:30	11:14
AD	F	Val/Met	e3/e4	73	16	22	70	11:51	11:33
AD	M	Val/Val	e3/e3	65	18	18	60	10:33	11:49
AD	F	Val/Val	e3/e4	64	16	21	67	10:59	11:01
AD	F			70	20	22	73	13:07	11:15
AD	F			66	14	22	77	10:08	10:42
AD	M			69	20	18	52	11:39	11:05
AD	M			54	18	22	65	11:44	11:01
AD	F	Val/Val	e4/e4	79	13	23	111	12:05	11:20

Abbreviations: BDNF, brain derived neurotrophic factor; APOE, apolipoprotein E; MMSE, mini-mental status exam; T2DM, type-2 diabetes mellitus; AD, Alzheimer's disease.

8.2.3 Transcranial Magnetic Stimulation

All parameters used in the study conformed to current recommended guidelines for the safe application of TMS endorsed by the IFCN (Rossi et al., 2009; Rossini et al., 2015). At the first visit, the integrated nTMS-EMG Nexstim system (eXimia NBS 4, Nexstim Plc, Finland) was used to anatomically identify the hand region and the most likely hotspot for FDI in the primary motor cortex, which was then marked on the participant's MRI to ensure consistent targeting throughout each TMS visit. The search for the hotspot and posterior measures of RMT (using both $mono_{PA}$ and biphasic pulses) and AMT (using biphasic pulses) followed the approach described for nTMS in *State of the Art* (*Chapter 4*) and following IFCN guidelines (Rossini et al., 2015). TMS was applied using a handheld $mono_{PA}$ figure-of-eight focal coil (Nexstim Plc, Finland). The hotspot and thresholds were reassessed at the second visit using the first visit hotspot as a reference.

In each visit the participants underwent to equivalent TMS testing protocols in the same order. First, block of single $mono_{PA}$-TMS pulses at 120% RMT provided a measure of unconditioned cortico-motor reactivity. Paired-pulse protocols included SICI, LICI and ICF using standard parameters (Valls-Solé et al., 1992; Kujirai et al., 1993). SICI and ICF consisted of a CP at 80% of RMT, a TP at 120% of RMT and an ISI of 3 and 12 ms, respectively. In LICI, CP and TP were 120% of RMT separated by an ISI of 100ms. Conditioned MEPs from SICI, LICI, and ICF blocks were averaged and expressed as the percent change from the unconditioned block. Detailed information of the physiology and basic protocols of single- and paired-pulse TMS can be found in *Methodology* (*Chapter 4 – Sections 4.2 and 4.3*). Paired-pulse measures could not be performed in two participants in whom RMT exceeded 83% of maximum stimulator output. Blocks of single and pairs of TMS pulses were separated by a randomized 5000-6000 ms interval to minimize train effects. Each block consisted of 50 trials and individual MEP amplitudes > 2.5 SD from the mean were excluded.

After the paired-pulse TMS neuronavigated iTBS was applied to participants using a handheld passive-cooling fluid-filled figure-of-eight coil (MCF-B65; 75 mm outer wing diameter) attached to a MagPro X100 stimulator (MagVenture A/S, Denmark). Intensity was 80% of AMT. The pattern was a two-second train of biphasic bursts (three pulses at 50 Hz) repeated every 200 ms (30 pulses per train). Trains were repeated 20 times with an eight-second inter-train interval (600 pulses, 192 seconds). As aforementioned in *Methodology* (*Chapter 4 – Section 4.4*) this protocol has been shown to potentiate cortico-motor reactivity for up to 60 minutes in healthy individuals (Huang et al., 2004; Wischnewski & Schutter, 2015).

Prior to iTBS, participants received three blocks of 30 single TMS pulses at 120% RMT using a hand-held bi_{AP-PA} figure-of-eight coil (Nexstim Plc). Cortico-motor reactivity was reassessed in blocks of 30 bi_{AP-PA} TMS pulses at 5, 10, 20, 30, 40, and 50 min post-iTBS. The peak-to-peak amplitude of each recorded MEP was measured automatically. For each block, individual MEPs > 2.5 SD from the mean were excluded. All 90 pre-iTBS trials were averaged as a measure of baseline cortico-motor reactivity. MEP trials were averaged for each post-iTBS block and expressed as the percent change from baseline. Due to complications, MEPs were not obtained at 30-min post-iTBS in two participants and 50-min post-iTBS in one participant. In those participants, the corresponding time-point from the other visit was therefore excluded from subsequent analysis.

8.2.4 Statistical Analyses

Neurophysiological data included three motor thresholds ($mono_{PA}$ and bi_{AP-PA} RMT and biphasic AMT; expressed as percent of MSO), two measures of cortico-motor reactivity (unconditioned MEPs elicited with the monophasic coil that was used to assess the effects of the paired-pulse paradigms and baseline MEPs elicited with the biphasic coil that were used to assess the impact of iTBS), three paired-pulse measures (SICI, LICI, ICF; with the average

amplitude of the conditioned MEPs expressed as the percent change the amplitude of unconditioned MEPs), and the six post-iTBS time-points (Post05, Post10, Post20, Post30, Post40, and Post50; with the average amplitude of MEPs from each time-point expressed as the percent change from the pre-iTBS baseline average). From the iTBS modulation, three further measures of plasticity were calculated: the maximum facilitation (Max+), or greatest change in MEP amplitude across all six time-points; the summed area under-the-curve for the first 20-min post-iTBS (AUC_{0-20}), corresponding to the period of peak effect in neurotypical individuals (Wischnewski & Schutter, 2015); and the summed area under-the-curve across all post-iTBS time-points (AUC_{0-50}). The area under-the-curve was calculated as the summed products of the average % of change in MEP amplitude at two consecutive time-points and the time in minutes between them.

For all neurophysiological measures, ICCs were calculated between the two visits to assess test-retest reliability using the ICC(A,1) formula (McGraw & Wong, 1996). The ICCs were calculated for all subjects together and for each group (AD, T2DM, controls) separately using MATLAB using the Statistics Toolbox (Release 2015b, The MathWorks, Inc., Natick, MA, USA, www.mathworks.com). In this study we followed the reliability classification which is most commonly adopted in TMS literature, described by Portney and Watkins (Portney & Watkins, 2009). ICC values were interpreted as high (ICC ≥ 0.75), moderate (0.5 ≤ ICC < 0.75), low (0.25 ≤ ICC < 0.5) or very low to none (ICC < 0.25).

As we already mentioned in statistics description of the previous study (*Chapter 7 – Section 7.3*) reliability coefficients, such as the ICCs, can be used to adjust effect sizes (Baugh, 2002; Wright, 2014). Using that same approach, we calculated adjustments for each measure with a hypothetical Cohen's *d* effect size of 0.5, which corresponds to a within-subjects change of half a standard deviation, and is considered a medium effect size (Cohen, 1992). Only positive ICCs values were used to adjust for effect sizes given the nature of the calculations.

To investigate factors associated with intra-individual variability, additional analyses were performed in JMP Pro (v12.1.0, http://www.jmp.com) using a normal distribution and a two-tailed 95% confidence interval. Given the exploratory nature of these analyses, individual *p*-values were not adjusted for multiple comparisons and should be interpreted accordingly. For between-groups comparisons, the sample sizes in the present study provided 0.80 power to detect a medium effect size (Cohen's d = 0.54). The first set of analyses concerned correlations between variables that were collected at each visit and thus were performed using the net difference between Visits A and B (Δ_{B-A}) so that the direction of change between visits was taken into consideration. These analyses included: [1] how differences in baseline MEP amplitude relate to differences in RMT (for both $mono_{PA}$ and bi_{AP-PA} pulses); [2] how differences in SICI, LICI, and ICF relate to differences in unconditioned monophasic MEP amplitudes and RMT; and [3] how differences in post-iTBS measures relate to differences in baseline biphasic MEP amplitudes, RMT, and AMT. The second set of analyses concerned factors, such as *Group*, *Gender*, *Age*, *Inter-Visit Interval*, and *BDNF* and *APOE* polymorphisms, that were assessed only once per participant. Multiple linear regression analyses were performed on the absolute value of the inter-visit difference ($|\Delta_{B-A}|$) to account for the amount of change between visits in either direction.

8.3 Results

Data on motor thresholds, baseline cortico-motor reactivity measures, changes in MEP amplitude from the paired-pulse TMS and post-iTBS plasticity measures are shown in ***Table 8.2***.

Table 8.2. Neurophysiological Measures.

	Visit-A	Visit-B	Δ_{B-A}	$\lvert\Delta_{B-A}\rvert$
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Motor threshold (% MSO)				
RMT monophasic	62.0 ± 12.9	63.9 ± 13.3	1.9 ± 4.7	4.00 ± 3.1
RMT biphasic	44.3 ± 11.6	43.9 ± 10.1	-0.4 ± 5.5	3.91 ± 3.8
AMT biphasic	43.9 ± 11.9	43.7 ± 10.1	-0.2 ± 6.7	4.38 ± 5.1
Baseline MEPs (mV)				
Monophasic	0.9 ± 0.9	0.9 ± 1.0	0.0 ± 0.8	0.49 ± 0.6
Biphasic	1.4 ± 1.0	1.3 ± 1.1	-0.0 ± 1.1	0.76 ± 0.8
Paired-pulse (%Δ)†				
SICI	-21.6 ± 65.7	-12.3 ± 60.0	9.3 ± 59.5	41.04 ± 43.5
LICI	-49.6 ± 76.2	-67.2 ± 49.0	-17.6 ± 72.1	34.80 ± 65.3
ICF	123.2 ± 204.5	143.4 ± 347.0	20.2 ± 372.5	164.50 ± 333.5
Post-iTBS (%Δ)††				
5 min post-iTBS	9.8 ± 48.0	25.9 ± 70.1	16.1 ± 76.6	55.44 ± 54.5
10 min post-iTBS	12.0 ± 47.3	9.8 ± 49.0	-2.2 ± 65.7	46.95 ± 45.3
20 min post-iTBS	-12.0 ± 43.2	15.2 ± 56.2	27.2 ± 62.7	51.35 ± 44.5
30 min post-iTBS	-1.5 ± 44.1	6.8 ± 57.9	8.3 ± 65.9	46.54 ± 46.7
40 min post-iTBS	9.5 ± 54.6	22.8 ± 101.3	13.3 ± 95.7	66.65 ± 69.0
50 min post-iTBS	-5.0 ± 49.8	15.5 ± 83.1	20.5 ± 92.0	59.16 ± 72.7
Max. Facilitation	50.6 ± 51.6	87.5 ± 96.7	36.9 ± 93.3	62.13 ± 78.3
*AUC (%Δ*time)*				
0-20 min post-iTBS	78.4 ± 683.9	278.6 ± 823.4	200.3 ± 936.9	687.1 ± 657.9
0-50 min post-iTBS	5.6 ± 1634.6	721.1 ± 2456.7	762.6 ± 2379.2	1728.4 ± 1783.2

Abbreviations: Δ_{B-A} = net inter-visit difference; $\lvert\Delta_{B-A}\rvert$ = absolute inter-visit difference; RMT = resting motor threshold; AMT = active motor threshold; MEPs = motor evoked potentials; SICI = short intracortical inhibition; LICI = long intracortical inhibition ; ICF = intracortical facilitation. † Percent change from monophasic baseline; †† Percent change from biphasic baseline.

8.3.1 Reliability of Neurophysiological measures

Figure 8.1 shows coefficients for all measures and all groups. ICC were classified following criteria for categorizing reproducibility in neurophysiological assessments mentioned in *State of the Art* (*Chapter 2 – Section 2.5*) (Portney & Watkins, 2009).

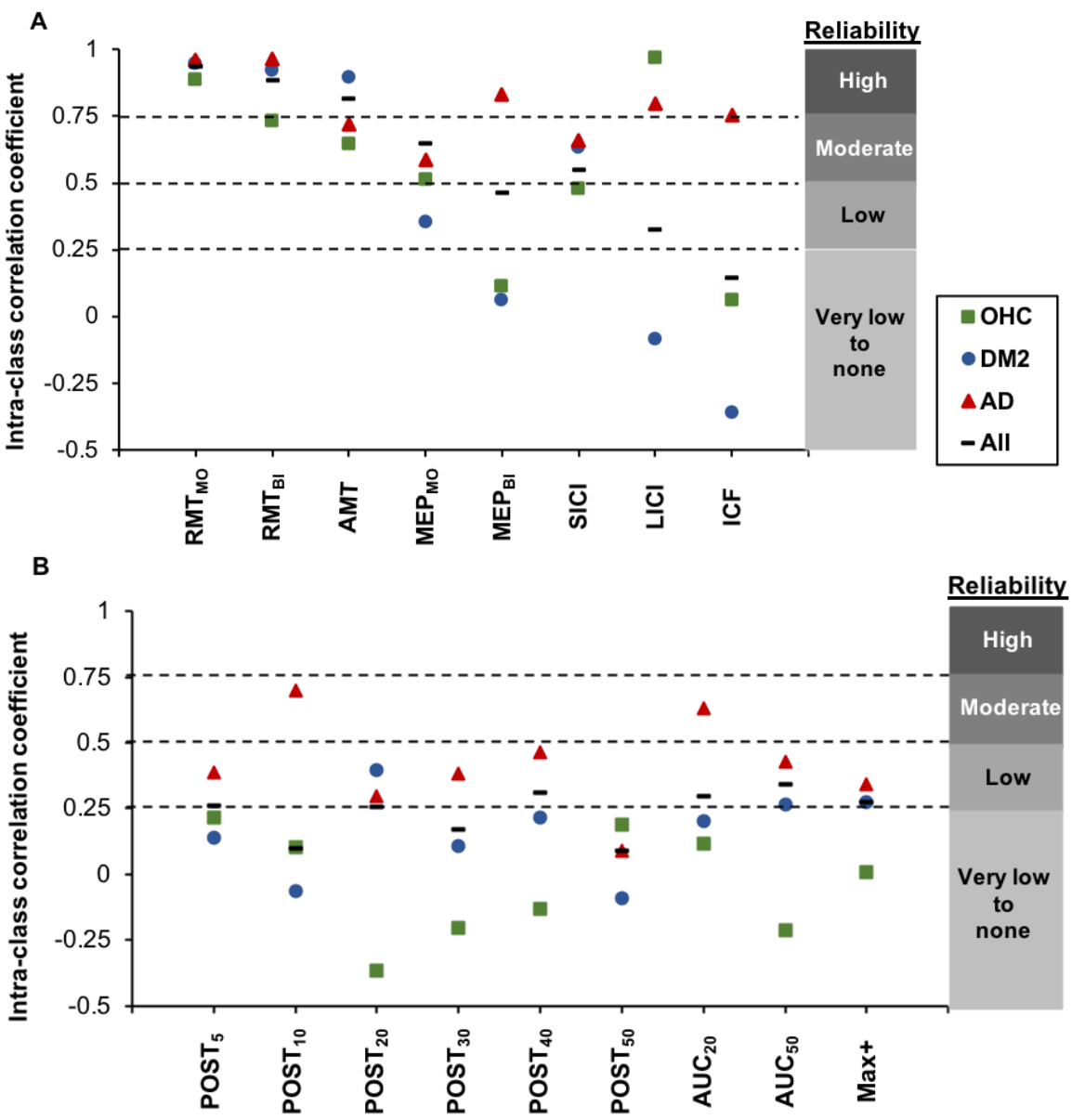

Figure 8.1. Reproducibility of TMS measures across groups.

Reproducibility of TMS measures across groups. The Intra-class correlation coefficient (ICC, y-axis) was calculated as an index of reliability for each TMS-based measure (x-axis); A, for single- and paired-pulse TMS measures and B for post theta burst (TBS) measures. ICCs were calculated for all subjects (solid line marker) as well as for each group: Alzheimer's disease (AD; red triangle marker); type-2 diabetes mellitus (T2DM; green circle marker); and non-AD/non-T2DM controls (blue square marker). *Abbreviations:* AMT, active motor threshold; AUC, area under-the-curve; BI, biphasic waveform; ICF, intracortical facilitation; LICI, long intracortical inhibition; Max+, maximum facilitation; MEPs, motor evoked potentials; MO, monophasic waveform; POST, minutes post-TBS; RMT, resting motor threshold; SICI, short intracortical inhibition.

Considering all groups combined, the three motor thresholds had high reproducibility (ICC's > 0.81). For baseline MEPs elicited at 120% of RMT, monophasic (ICC = 0.65) pulses showed moderate reliability while the biphasic pulses (ICC = 0.47) had low reliability. Among the paired-pulse measures, reproducibility was low to moderate for LICI (ICC= 0.33) and SICI (ICC = 0.55), respectively. While ICF was not reproducible (ICC = 0.14). All post-iTBS measures demonstrated low reproducibility (ICC's = 0.26-0.34); except for Post10 (ICC = 0.10), Post30 (ICC = 0.17) and Post50 (ICC = 0.09), which were not reproducible.

Considering each group separately, ICCs tended to be higher for the AD group than for T2DM and controls. In particular, the AD group demonstrated high reproducibility for resting thresholds (ICC's = 0.96), biphasic MEPs (ICC = 0.83), LICI and ICF protocols (ICC's > 0.75). Further, reproducibility in AD was moderate for AMT (ICC = 0.72), monophasic MEPs (ICC = 0.59) and SICI (ICC = 0.66). For the remaining post-iTBS measures the reliability was low to none (ICC's = 0.09-0.47) with the exception of Post10 (ICC = 0.70) and $AUC_{0\text{-}20}$ (ICC = 0.63) that showed moderate reliability values. By comparison, both controls and T2DM individuals showed low to no reproducibility in most of the TMS measures that were analyzed (ICCs = -0.37-0.48). Positive outliers were SICI in T2DM (ICC = 0.63), and monophasic MEPs (ICC = 0.51) and LICI in controls (ICC = 0.97) that showed moderate to high reproducibility, respectively. Reproducibility coefficients together with hypothetical effect sizes adjusted for these ICCs in all subjects and in the three separate cohorts are shown in ***Table 8.3***.

Table 8.3. Reliability coefficients and corresponding adjusted effect and sample sizes.

	ICC				Reproducibility-adjusted Cohen's d (0.5)				Additional *n* required			
	All subjects	Controls	T2DM	AD	All subjects	Controls	T2DM	AD	All subjects	Controls	T2DM	AD
Motor threshold (% MSO)												
RMT monophasic	0.93	0.89	0.95	0.96	0.491	0.485	0.493	0.495	1	3	1	1
RMT biphasic	0.89	0.73	0.92	0.96	0.484	0.461	0.489	0.495	3	6	1	0
AMT biphasic	0.82	0.65	0.90	0.72	0.474	0.445	0.486	0.458	4	7	1	6
Baseline MEPs (mV)												
Monophasic	0.65	0.51	0.36	0.58	0.446	0.419	0.381	0.434	7	13	23	11
Biphasic	0.46	0.11	0.06	0.83	0.409	0.284	0.241	0.476	15	69	105	3
Paired-pulse (%Δ)[†]												
SICI	0.55	0.48	0.63	0.66	0.427	0.412	0.443	0.448	11	15	9	7
LICI	0.33	0.97	-0.09	0.80	0.373	0.496	-	0.471	26	0	-	4
ICF	0.14	0.06	-0.36	0.76	0.302	0.243	-	0.464	56	105	-	6
Post-iTBS (%Δ)[††]												
5 min post-iTBS	0.26	0.22	0.14	0.39	0.351	0.335	0.300	0.390	33	36	56	20
10 min post-iTBS	0.10	0.11	-0.07	0.70	0.276	0.279	-	0.455	69	69	-	7
20 min post-iTBS	0.26	-0.37	0.40	0.30	0.351	-	0.393	0.364	33	-	20	29
30 min post-iTBS	0.17	-0.20	0.11	0.38	0.316	-	0.282	0.389	45	-	69	20
40 min post-iTBS	0.31	-0.13	0.22	0.47	0.368	-	0.335	0.409	26	-	36	15
50 min post-iTBS	0.09	0.19	-0.09	0.09	0.269	0.324	-	0.267	76	45	-	76
Max. Facilitation	0.27	0.01	0.28	0.34	0.356	0.146	0.357	0.378	29	317	29	23
*AUC (%Δ*time)*												
0-20 min post-iTBS	0.30	0.12	0.20	0.63	0.364	0.286	0.329	0.443	29	62	41	9
0-50 min post-iTBS	0.34	-0.21	0.27	0.43	0.377	-	0.354	0.401	23	-	33	18

Only positive Intra-class correlation coefficients (ICCs) values were used to adjust effect sizes. Abbreviations: AMT, active motor threshold; AUC, area under-the-curve; ICF, intracortical facilitation; LICI, long intracortical inhibition; MEPs, motor evoked potentials; RMT, resting motor threshold; SICI, short intracortical inhibition.

8.3.2 Relationships between the net differences of neurophysiological measures

For measures assessed with a $mono_{PA}$ pulse, there were no significant relationship between the Δ_{B-A} of baseline MEP amplitudes and the Δ_{B-A} of RMT, $R_{31} = 0.01$, $p = 0.934$. Similarly, there were no significant relationships between the Δ_{B-A} of any of the paired-pulses measurements with the Δ_{B-A} of either RMT or baseline MEP amplitudes ($|R|$'s < .27, p's > 0.13).

When using a bi_{AP-PA} pulse, the Δ_{B-A} of baseline MEP amplitudes was significantly correlated with the Δ_{B-A} of RMT, $R_{34} = -0.35$, $p = 0.035$. Specifically, a 1% MSO increase in the net difference of RMT was associated with a 70-µV decrease in the net difference of baseline MEP amplitude (***Figure 8.2A***). Further, the Δ_{B-A}'s for all iTBS plasticity measures except Post30 and Post40 were significantly correlated with the Δ_{B-A} of bi_{AP-PA} baseline MEPs amplitudes (R's < -.36, $p < 0.04$). In all cases, an increase in the net difference of baseline MEP amplitudes was associated with a decrease in the inter-visit difference of post-iTBS facilitation. This relationship was most apparent for AUC_{0-20}, where a 1-mV increase in the inter-visit difference of baseline MEP amplitude was associated with a 390 (%Δ*min) decrease in the net difference of the AUC (***Figure 8.2B***). By contrast, there were no significant relationships between the Δ_{B-A} of any of the iTBS plasticity measures with the Δ_{B-A} of either RMT or AMT ($|R|$'s < 0.32, p's > 0.07). These results indicate that as much as 23% of the visit-to-visit variability in iTBS plasticity measures can be accounted for by the variability in the baseline MEP amplitude, which in turn is impacted by the variability in RMT.

In the T2DM subjects, blood glucose levels did not differ significantly between visits (p > 0.1) and no significant relationships were observed between changes in blood glucose levels and changes in any TMS measure between visits (p's > 0.2).

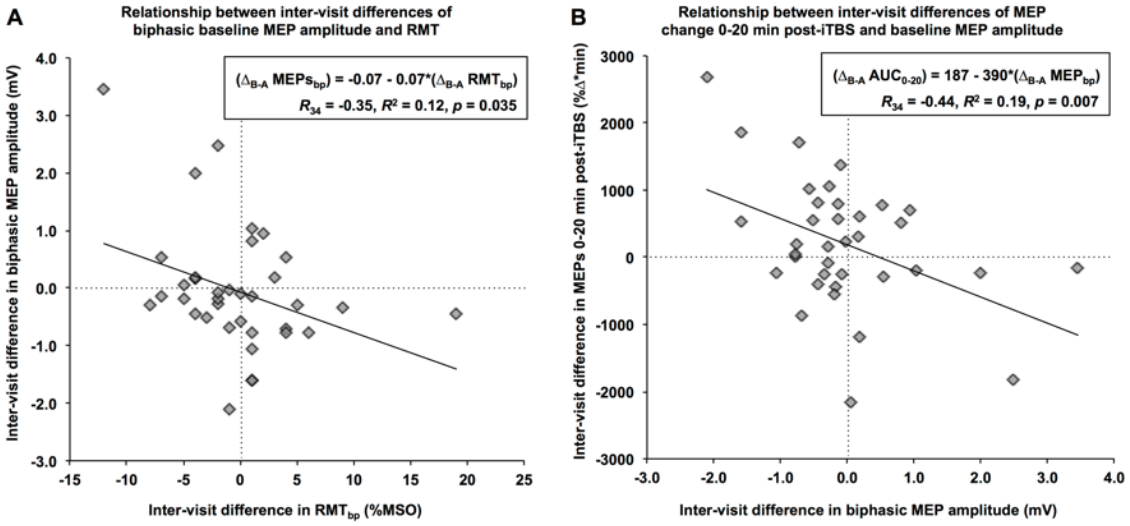

Figure 8.2. Relationships between the net difference of neurophysiological measures.
(**A**) Using biphasic pulse, an increase in RMT of 1% of maximum stimulator output from Visit-A to Visit-B (*x*-axis) was associated with a decrease of 0.07 mV in baseline MEP amplitude over the same period (*y*-axis). (**B**) An increase of 1 mV in baseline MEP amplitude from Visit-A to Visit-B (*x*-axis) was associated with an inter-visit decrease of 390 % Δ*min in the MEP change during the first 20 min (*y*-axis).

8.3.3 Analyses of the absolute difference between visits

Controlling for the *Inter-Visit Interval*, as well as the *Age* and *Gender* of participants, the linear model yielded no difference between groups in the $|\Delta_{B\text{-}A}|$ of any neurophysiological measure (*F*'s < 2.2, *p*'s > 0.13). These results indicate that the absolute amount of change between visits in motor thresholds, as well as baseline reactivity, paired-pulse TMS, and plasticity measures were equivalent across AD, T2DM and control participants at the .05 level.

The multiple regression analyses did show a significant relationship between the $|\Delta_{B\text{-}A}|$ of monophasic MEPs and *Age*, controlling for *Group*, *Gender*, and *Inter-Visit Interval* ($F_{1,1}$ = 5.62, *p* = 0.025). Specifically, a one-year increase in participant *Age* was associated with a 0.03 mV

increase in the absolute visit-to-visit difference in the amplitude of bi_{AP-PA} MEPs. Similarly, there was a significant relationship between the $|\Delta_{B-A}|$ of Post05 facilitation and *Inter-Visit Interval*, controlling for *Group*, *Gender*, and *Age* ($F_{1,1} = 6.42$, $p = 0.017$). Specifically, a one-day increase in the interval between visits was associated with a 0.03 mV decrease in the absolute inter-visit difference in the %Δ in MEP amplitudes at Post05. None of the other relationships were significant (F's < 4.0, p's > 0.05) (***Figure 8.3A***).

Regarding the influence of genetic polymorphisms, the multiple regression analyses demonstrated there was a significant effect of *BDNF* status on the $|\Delta_{B-A}|$ of several post-iTBS measures after controlling for *Group* (***Figure 8.3B***). Specifically, intra-individual variability was higher for *BDNF*-Met carriers than *BDNF*-Val homozygotes for Post05 ($F_{1,1} = 6.76$, $p = 0.017$) and Post50 ($F_{1,1} = 6.79$, $p = 0.017$), and AUC_{0-20} ($F_{1,1} = 4.99$, $p = 0.037$). By comparison, there was no significant effect of *APOE* status on the $|\Delta_{B-A}|$ of any of TMS measures (F's < 2.8, p's > 0.1).

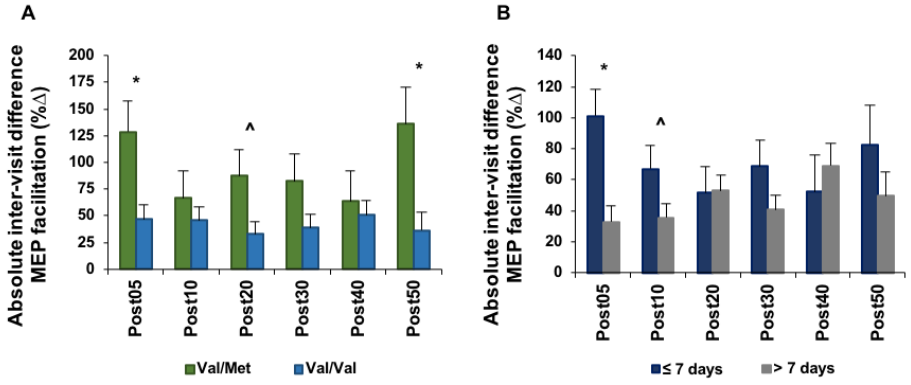

Figure 8.3. Additional sources of Variability.

(**A**) Impact of BDNF polymorphism on the reliability of iTBS after-effects. Controlling for Group, the absolute difference in MEP facilitation (*y*-axis) tended to be higher across all post-iTBS time-points (x-axis) in Val-Met carriers (green) than Val/Val homozygotes (light blue). (**B**) Impact of inter-visit duration on the reliability of iTBS after-effects. Controlling for Group, Age and Gender, the absolute difference in MEP facilitation (*y*-axis) tended to be higher across all post-iTBS time-points (*x*-axis) in subjects that received their second visit within 7 days (dark blue) than those whose second visit occurred greater than 7 days after the first (grey). * $p < 0.05$, ^ $p < 0.1$.

8.4 Discussion

We have previously discussed that the potential of TMS-based assessments to provide meaningful insights into human neurophysiology is constrained by its variability. In particular, the intra-individual variability of a given TMS measure could reduce its sensitivity to detect meaningful changes over time or in responses to an intervention. *State of the Art* (*Chapter 2 – Section 2.5*) summarizes the studies that have investigated the reliability of different TMS measures thus far and some of the common causes of their variability. However, as TMS is increasingly applied in different neuropsychiatric conditions, it is crucial to evaluate its reproducibility not only in terms of the technical parameters but also elucidate how target populations could be affected or may or may not introduce further variability to the measure. The current study offers the first direct analysis of reproducibility of single- and paired-pulse TMS, and patterned repetitive TMS in older healthy adults and those with impaired cognition or glucose metabolism. The results show that reproducibility varies considerably across measures and populations. Motor thresholds remain the gold standard in test-retest reliability; SICI and LICI tended to be more reproducible than ICF, though variability in LICI and ICF differed considerably across groups. Lastly, measures of LTP-like plasticity from iTBS were among the least reproducible, especially for older healthy and diabetic individuals.

Two recent studies in young healthy individuals have reported higher intra-individual variability in the response to iTBS (Hinder et al., 2014; Schilberg et al., 2017). The present results, based on data from healthy older adults and those with either impaired cognition or glucose metabolism, are more-or-less consistent with those reports in young adults and suggest that variability in the after-effects of iTBS remains a significant challenge to its use as a biomarker for the efficacy of neuroplastic mechanisms across the lifespan. In *Chapter 2 – Section 2.5* we have also reviewed some of the factors that can influence the efficacy of TBS and thus increase intra-individual variability such as prior exercise (McDonnell et al., 2013), ongoing voluntary activity

(Iezzi et al., 2008), and other state-dependent effects (Silvanto & Pascual-Leone, 2008). Other factors that are relevant for the present work such as BDNF polymorphisms and baseline cortico-motor reactivity are further discussed below. Some factors could be disease-specific, such as fluctuations in blood glucose levels in T2DM, though importantly glucose levels (within the range of 80-200 mg/dL specified *a priori*) were not found to influence variability in the present study. Interestingly, the AD group showed numerically higher reproducibility coefficients for nearly all measures, including RMT, SICI and iTBS after-effects, which several studies in AD have shown to be abnormal and/or predictive of disease severity or response to treatment (Liepert, Bär, Meske, & Weiller, 2001; Di Lazzaro et al., 2004; Koch et al., 2012, 2016; Brem, Atkinson, Seligson, & Pascual-Leone, 2013; Balla, Maertens de Noordhout, & Pepin, 2014). It is possible, however unlikely, that some aspect of the Sham treatment (e.g., daily study visits or interaction with study staff) that the AD group underwent had some stabilizing effect on their neurophysiology. This possibility could be investigated further by conducting test-retest assessments in a similar AD cohort over a similar timeframe that did not include significant changes to their regular schedule. A more likely explanation is that the same pathological processes that cause certain measures to be abnormal in AD also exert a stabilizing effect on TMS measures. It is important to highlight that the responses of our AD cohort were aligned with those in previous studies where a reduction in LTP-like plasticity following iTBS is shown (and in some cases absence of LTP-like plasticity or even conversion to a LTD-like response) (Koch, 2010; Koch et al., 2012; Di Lorenzo et al., 2016). This lack of response together with a greater reliability compared to those study participants that were cognitively intact (i.e. older healthy controls and T2DM) could reflect pathological changes in the brains of AD patients that reduce state-dependent effect and more neurophysiological rigidity less likely to change after the stimuli. In any case, the relatively high reproducibility of most TMS measures in AD appears to validate their use as surrogate biomarkers of AD cortical pathology (Freitas et al., 2011).

As explored in the previous set of experiments (*Chapter 7*), ICC's can be used to adjust effect sizes trying to provide a more realistic estimate of the sample size required to observe the desired effect in relation to the reproducibility of the measure being tested. ***Table 8.3*** shows adjustments to a hypothetical Cohen's *d* of 0.5, corresponding to a change of half a standard deviation, and the sample sizes required to detect attenuated effects for each of the measures in the current analysis. The results of the present analysis can thus be used to more accurately plan for future studies using TMS-based neurophysiological measures as prognostic biomarkers in older healthy, diabetic, and AD populations.

8.4.1 Variability in baseline MEP and its role in post-iTBS variability

The reproducibility of bi_{AP-PA} MEP amplitude (ICC = 0.46) was noticeably lower than that of biphasic RMT (ICC = 0.89), when considering all subjects together. Given that MEPs were assessed using 120% of RMT, there appear to be factors that do not impact RMT but do add variability to batches of MEPs elicited at suprathreshold intensities.

Epidural recordings of cortico-spinal volleys in conscious humans receiving TMS over motor cortex have shown that depending on its shape, current direction, and intensity, a TMS pulse can result in direct depolarization of the PTN cell (D-waves) and/or indirect depolarization through local circuits of interneurons (I-waves) (Burke et al., 1993) (more detailed information about these studies can be found on *State of the Art*). Also in *State of the Art* (*Chapter 2 – Section 2.3*) we have discussed how at threshold intensities the second half of the biphasic pulse (posterior-anterior in the present study) contributes primarily to the activation of cortical components, while at suprathreshold intensities there is increasing influence of the first half of the pulse (anterior-posterior in the present study) (Di Lazzaro et al., 2003; A. Barker, 2017) contributing to MEP amplitude. Moreover, from the results of this study, the use of $mono_{PA}$ pulse waveforms, which primarily elicit early I-waves, yields higher reproducibility in measures of

cortico-motor reactivity over biphasic stimulation, which as just argued elicits a more complex pattern of D-waves, and early and late I-waves depending on the intensity of stimulation (Di Lazzaro et al., 2003).

Additionally, in the previous experiments of this thesis (Chapter 7) we have proven the importance of the technical/physical TMS parameters (such as pulse waveforms and current direction) and their influence in both the efficacy and reliability of baseline MEP amplitudes in a different cohort.

There is at least some theoretical evidence that biphasic TMS pulses might be less effective than $mono_{PA}$ at probing the neuromodulatory effects of TBS (Di Lazzaro & Rothwell, 2014).

At this point is worth emphasizing that post-iTBS data in our study was elicited using bi_{AP-PA} pulses. Knowing that different pulse waveforms and current directions translate the activation of distinct cortical circuits, future studies should directly explore how the effect size and reproducibility of single-pulse, paired-pulse, and TBS-based TMS measures are influenced by physical TMS parameters such as pulse shape and duration, and induced current direction relative to the motor cortex in this populations. However, to our knowledge, this has never been directly investigated. This may help improving the reliability of the measures as well as helping understanding the undergoing cortical processes.

Moreover, the inter-visit change in biphasic baseline MEP amplitudes was inversely related to that of biphasic RMT, suggesting that input-output curve (i.e., the relationship between TMS intensity and MEP size, see *Methodology*, *Chapter 4 – Section 4.2* for a schematic representation of an input-output curve) itself varies across visits. Regardless, the relatively low reproducibility of baseline MEP amplitudes is an area of concern given that it is the basis on which post-iTBS measures are derived. Furthermore, a significant portion of the inter-visit variance in post-iTBS measures is accounted for by visit-to-visit difference in baseline MEP amplitudes. The high variability of the biphasic baseline responses together with the poor reliability of post-iTBS

measurements are consistent with a recent study showing that variability in MEPs within a session is predictive of the response to cTBS (Hordacre, Ridding, & Goldsworthy, 2015). Furthermore, the present results imply that improving the consistency of baseline measures (within and across sessions) would decrease the variability of post-iTBS measures as well. Given that changes in input-output curves might contribute to the changing relationship between RMT and suprathreshold MEP amplitudes, future studies should also explore whether the reproducibility of these measures could be improved by choosing stimulation intensities based on individual stimulus-response curves rather than a fixed percent of RMT.

The use of neuronavigation has been shown to increase the consistency of MEPs (Julkunen et al., 2009). However, even with neuronavigation, handheld TMS remains prone to slight deviations in the position, orientation, and inclination of the TMS coil. Robot arms, such as the TMS Robot (Axilum® Robotics, Strasbourg) have been shown to improve the consistency of trial-to-trial MEPs over handheld TMS (Foucher et al., 2012; Ginhoux et al., 2013). Typically, MEP trials are elicited with individually spaced TMS pulses at a specific frequency range (e.g., 5000-6000 ms in the present study) with some random jitter incorporated to reduce the likelihood of train effects. Several recent studies combining TMS with concurrent EEG have highlighted to role of pre-stimulus oscillatory activity on cortico-motor excitability. Mäki and Ilmoniemi (Mäki & Ilmoniemi, 2010) demonstrated that MEP amplitudes are inversely correlated with the amplitudes of pre-stimulus midrange-beta oscillations (15-18 Hz) over the stimulated motor cortex. Similarly, Iscan and colleagues (Iscan, Nazarova, Fedele, Blagovechtchenski, & Nikulin, 2016) showed that the variability of pre-stimulus power in the upper alpha band (10-12 Hz) was predictive of variability in ICF trials. Alternatively, Berger and colleagues (Berger, Minarik, Liuzzi, Hummel, & Sauseng, 2014) suggest that the instantaneous phase of EEG oscillations across a range of frequencies is more predictive of MEP amplitude than spectral power. Together, these studies suggest that technological advances that allow for closed-loop systems to trigger TMS pulses timed to real-time EEG rhythms should result in more consistent MEPs. While these approaches

offer the potential to improve the trial-to-trial consistency of MEPs, whether they would translate to greater reproducibility across visits remains to be explored.

8.4.2 Impact of Age and Inter-Visit Interval

While the multiple regression analyses did not show any significant difference between groups in terms of the absolute difference of the measures, participant *Age* was significantly related to the absolute difference of monophasic baseline MEP amplitudes and *Inter-Visit Interval* was significantly related to the absolute difference in Post05 facilitation, controlling for other factors such as *Group* and *Gender*. These results must be interpreted cautiously given the potential for Type-2 error in the present analysis. That the variability in baseline MEPs increases with age is not particularly surprising given that the activation of motor system becomes more complex through central compensatory mechanisms (Ward & Frackowiak, 2003) (*Chapter 2 – Section 2.4* deepens on the relationship between age and the motor system); however, its influence is not easily controlled, especially if the focus of the study is aging. It is more surprising that immediate iTBS after-effects would be more consistent with greater time between visits. One possibility is that visits repeated under shorter intervals might be influenced by the iTBS from the previous visit, a phenomenon known as *metaplasticity*. It has been shown that the neuromodulatory effects of rTMS increase with consecutive daily application (Maeda, Keenan, Tormos, Topka, & Pascual-Leone, 2000; Valero-Cabré, Pascual-Leone, & Rushmore, 2008) . Moreover, these metaplastic effects and state-dependent interactions may be modulated by age (Opie, Vosnakis, Ridding, Ziemann, & Semmler, 2017) or neuropsychiatric disorders such as autism and Fragile X syndrome (Oberman et al., 2016). While the impact of multiple sessions separated by more than 24 hours has not been well explored, a single application of iTBS was shown to alter the expression of GABA-precursor enzyme GAD67 for up to 7 days in the neocortex of rats (Trippe, Mix, Aydin-Abidin, Funke, & Benali, 2009), suggesting the window for metaplastic

effects might be longer than previously understood. Indeed, a follow-up analysis of the current data found that the absolute difference of Post05 facilitation was higher between visits conducted within 7 days than those separated by more than a week (***Figure 8.3B***).

8.4.3 Influence of BDNF polymorphisms

The multiple regression analyses showed that the absolute difference of several post-iTBS measures was higher in subjects with a *BDNF*-Met allele. While the generalizability of these findings is limited by the small sample size, they nonetheless provide insight into the debate over the role of *BDNF* polymorphisms in shaping the effects of neuromodulation. Several studies have reported a reduced impact of repetitive TMS in Met carriers (Cheeran et al., 2008; Cirillo et al., 2012; Lee et al., 2013; Chang et al., 2014; Di Lazzaro et al., 2015), still others have reported no difference (Li Voti et al., 2011; Mastroeni et al., 2013). The current results on a subset of our sample suggest that this divergence in the literature may be due to the fact that the *BDNF*-Met allele leads to more variability in the response to neuromodulation rather than simply blunting its effects.

8.5 Conclusions

Motor thresholds remain the gold standard for reproducibility of any TMS measure as demonstrated by high ICC coefficients. Post-iTBS measures of LTP-like plasticity demonstrate low reproducibility by comparison. Reproducibility was higher in the AD group, possibly reflecting pathological rigidity of neurophysiological systems. A number of factors may contribute to the intra-individual variability of iTBS after-effects, including *BDNF* polymorphisms and variability in

baseline MEP amplitudes, from which post-iTBS measures are calculated. Future studies can use the ICC to adjust expected effect size and required sample size calculations.

Based on these conclusions, we offer the following recommendations for future studies to potentially reduce the intra-individual variability in TMS measures, especially in the iTBS induced modulation of cortico-motor reactivity. We note that these recommendations are based on exploratory analyses performed in a relatively small and heterogeneous group of subjects and further confirmatory studies are needed. (1) Waiting at least 7 days between repeated visits can reduce the probability of metaplastic effects, at least in healthy individuals. (2) Whenever possible, BDNF polymorphism should be taken into account, either by adding BDNF Met carrier status as a covariate, or by splitting the data into subgroups. (3) To reduce intra-individual variability in baseline MEP amplitudes and any resulting impact of this variability on post-iTBS measures, we recommend considering the use of a stimulation intensity derived from individual stimulus-response curves, rather than using a fixed percent of RMT.

9 Reliability measures in young and older healthy controls. A comparison between cohorts from the previous studies

Lastly, after investigating the effects of physical – technical TMS parameters and age-related diseases on the reliability of TMS reliability, we decided to retrospectively compare the reliability coefficients between our young and older healthy cohorts. This last analysis tries to elucidate if there might be possible differences in the reliability of common TMS single- and paired-pulse measures between those two groups of healthy participants.

As we already mentioned in *Chapter 2 – Section 2.4*, the previous concept of a static brain that only changes in the early stages of life or as a result of a disease seems to be overdue and the scientific community mostly concludes today that the brain continuously changes across lifespan from the moment we are born to the elderly ages.

In this last analysis of reliability, data on RMT, baseline MEP amplitude, and paired-pulse protocols with $mono_{PA}$ and $bi_{AP\text{-}PA}$ pulses was compared between the young healthy cohort from the first set experiments (*Chapter 7*) and 12 older healthy controls (6 males, mean ± SD age: 58.6 ± 9.1 years) that were both cognitively intact (MMSE ≥ 27) and non-diabetic (hemoglobin A1c < 6.2%) (*Chapter 8*). As previously mentioned, none of the participants had any unstable medical condition, drug intake or comorbidity.

As a reminder, surface EMG activity was recorded from the dominant hand's FDI and electrodes were placed in a belly-tendon montage with the ground electrode over the ipsilateral ulnar styloid process. Data consisted of RMT and baseline cortico-motor reactivity using both $mono_{PA}$ and $bi_{AP\text{-}PA}$ pulses and SICI, LICI, ICF using $mono_{PA}$ pulses only. Aside from the RMT, the protocols in the young and older populations differed slightly. However, both Nexstim and MagPro have similar coil windings and were configured to induce similar pulse waveforms and

current directions in the brain. MEP peak-to-peak amplitudes (mV) of the non-rectified signal were recorded for individual traces and percentage of change from baseline MEP amplitude was calculated for paired-pulse protocols. Participants were monitored for drowsiness and asked to keep their eyes open throughout the experiment. The ICC's, as previously mentioned, were calculated between the two visits to assess test-retest reliability using the ICC(A,1) formula (McGraw & Wong, 1996). The ICCs were calculated for young and older healthy controls groups depending on the waveform/current direction separately using MATLAB using the Statistics Toolbox (Release 2015b, The MathWorks, Inc., Natick, MA, USA, www.mathworks.com). In this study we followed the reliability classification which is most commonly adopted in TMS literature, described by Portney and Watkins (Portney & Watkins, 2009). ICC values were interpreted as high (ICC ≥ 0.75), moderate (0.5 ≤ ICC < 0.75), low (0.25 ≤ ICC < 0.5) or very low to none (ICC < 0.25).

The ICC data for RMT ($mono_{PA}$, bi_{AP-PA}), baseline MEP ($mono_{PA}$, bi_{AP-PA}), and SICI, LICI, ICF ($mono_{PA}$) were compared between the young and the older cohort using two-way mixed-effects *F* statistics.

The reliability coefficients for the different single- and paired-pulse TMS measures are represented in ***Figure 9.1***. RMT and LICI with $mono_{PA}$ pulses were both significantly more reliable among the older than the young controls ($p = .028$, and $p < .001$, respectively). The ICCs for other TMS measures were not significantly different between the two age groups (all p's > 0.060).

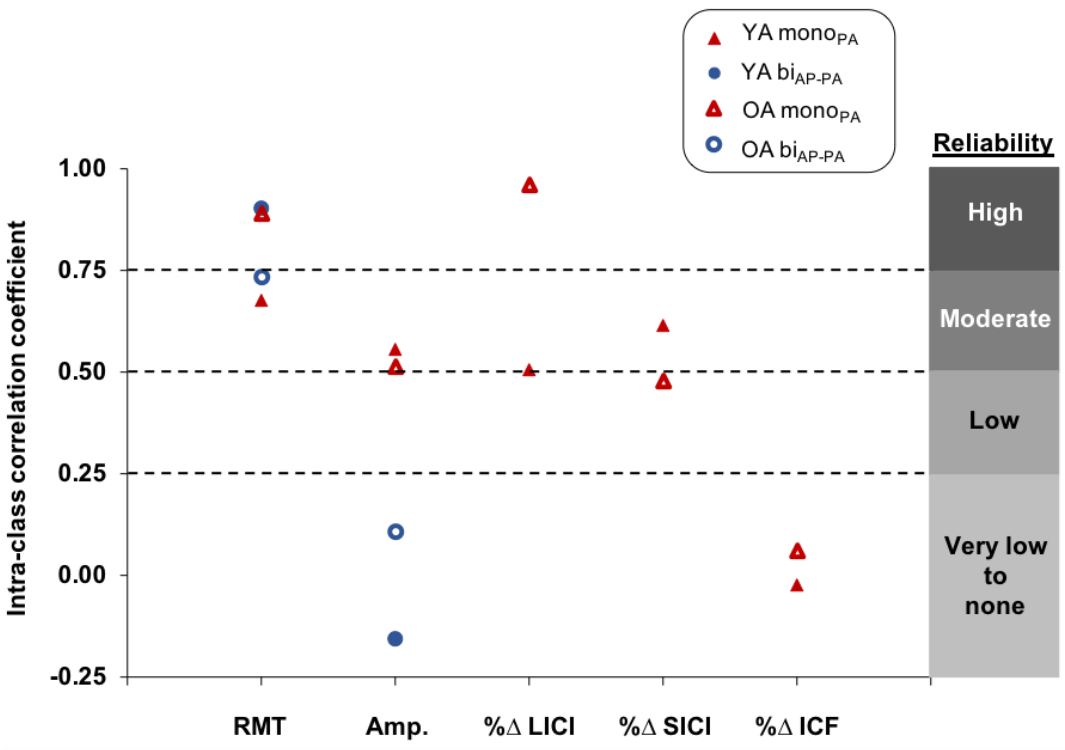

Figure 9.1. Reliability of single- and paired-pulse measures.
Intra-class correlation coefficients (ICCs) for the different TMS protocols performed with mono$_{PA}$ (monophasic posterior-to-anterior) and bi$_{AP-PA}$ (biphasic anterior-to-posterior—posterior-to-anterior) in young adults (YA, ages 18 – 35) and older adults (OA, ages 50 – 79). *Abbreviations: Amp.,* baseline MEP amplitude; *RMT,* resting motor threshold; %Δ LICI, long interval intracortical inhibition percentage of change from baseline; %Δ SICI, short interval intracortical inhibition percentage of change from baseline; %Δ ICF, intracortical facilitation percentage of change from baseline.

The ICCs of most TMS measures were reassuringly quite similar between the young and the older healthy controls from the two previous experiments. Interestingly, however, the RMT and LICI with mono$_{PA}$ pulses, which were the two most reliable TMS measures among the older controls in the study of reliability of TMS measures in aging and age-related (*Chapter 8*), were both significantly more reliable in the older than in the young adults.

The higher reliability of mono$_{PA}$ RMT in the older compared to young could be due to several factors: (1) the use of the participant's individual brain MRI for older adults may have

improved the consistency of localizing the motor hotspot between the two visits compared to using a brain MRI template for young controls in the present study. A more consistent localization of motor hotspot, in turn, may have improved the reproducibility of RMT; (2) alternatively, normal aging may lead to an increase in the rigidity of neurophysiological systems, which may in turn reduce the influence of state-dependent effects and other factors that contribute to the intraindividual variability in corticospinal excitability in younger adults.

The higher reliability of LICI among the older adults could be attributed to several factors: (1) Unlike young controls, most of the older showed nearly complete inhibition of MEPs after LICI, suggesting a floor effect. Such an effect seen in both visits and which bounds the data on one side, would have the impact of minimizing inter-visit variability; (2) There might be differences between young and older controls in the efficacy of intracortical inhibition, as indexed by LICI, due to age-related changes in the efficiency of $GABA_B$ synaptic transmission. While studies in rodents have suggested the overall efficacy of $GABA_B$-mediated inhibition decreases with aging (McQuail, Banuelos, LaSarge, Nicolle, & Bizon, 2012), the 100-ms inter-pulse interval may have been sub-optimal for the young adults relative to the older. (3) Finally, slightly different methods were used to measure paired-pulse effects among young and older cohorts: conditioned MEPs were measured in separate blocks for older but were intermixed in a pseudorandom order for young controls. It is, however, unclear why such a difference would affect LICI but not SICI or ICF. Unlike SICI and ICF, which are obtained with a narrow range of short ISIs, LICI is obtained with a wide range of longer ISIs. That wider range of ISIs can result in LICI, making it more likely that LICI-induced inhibition occurs with a particular ISI in a given subject. In contrast, the range of optimal ISIs for SICI and ICF may be narrower, making them less likely to result in similar effects in both age groups across visits, where there might be differences in the efficacy of intracortical inhibition mediated by GABAergic synaptic transmission. Future studies could investigate this further by conducting a response curve of LICI using different ISIs in younger and older participants.

SECOND BLOCK OF EXPERIMENTS: EFFECTS OF TRANSCRANIAL STATIC MAGNETIC STIMULATION ON MOTOR CORTEX EXCITABILITY AND BRAIN OSCILLATORY ACTIVITY

10 Effects of transcranial Static Magnetic Stimulation (tSMS) on motor cortex excitability and brain oscillatory activity in healthy subjects.

10.1 Introduction

Dynamic magnetic and electric fields have been used for decades to explore human brain function, brain physiology in health and disease, and have been proved to modulate the activity of the brain helping in the treatment of different diseases. The most known example of this is TMS.

Recently, several studies have found that moderate SMFs (i.e. magnetic fields between 1mT to 1T (Rosen, 2003) that do not change over time) also influence human cortical excitability. The use of SMFs as a NIBS tool has grown as a new and promising brain stimulation technique, further developing its potential for the modulation of brain cortical activity.

The exposure to tSMS for 10-15 min induces a reduction of TMS elicited MEPs of about 25% that outlast the intervention for several minutes and is negatively correlated with an increase

in RMT (Oliviero et al., 2011; Silbert et al., 2013) translating a decrease in motor cortex excitability due to the effects of the SMF.

After these initial studies, other research groups continued exploring the effects of tSMS on (1) motor cortex, performing different TMS inhibitory protocols, and (2) other cortical areas, such as somatosensory and visual. As a summary of the studies on tSMS effects previously mention in *State of the Art* (*Chapter 3*), we can presume that tSMS reduces cortical excitability through $GABA_A$-inhibitory cortical circuits that are involved in SICI. Nevertheless, the involvement of $GABA_A$-inhibitory circuits seems to be rather specific given that other protocols like SAI or LAI were not influenced by the SMFs. Hence, the physiological mechanisms of the induced inhibition of the motor cortex after tSMS are still unknown and additional studies are still needed in order to elucidate possible cortical circuits that are involved.

One way of deepening on the understanding of the cortical mechanisms of tSMS is by using different TMS waveforms and current directions. We know from Di Lazzaro's studies (Di Lazzaro et al., 2017) that different TMS waveforms and current directions may activate specific neural circuits related to the motor system (for further information read *State of the Art*, *Chapter 2*). Therefore, the aim of the present study was to deepen on the understanding on the tSMS-motor cortex interactions by using different waveforms and current directions when performing common TMS protocols to evaluate cortical excitability and the balance between facilitatory and inhibitory cortical networks.

The second aim intended to further investigate the effects of tSMS on the motor system by measuring the cortical oscillatory activity with EEG and relate the possible changes to the reduction in MEP amplitude seen with the TMS evaluation. Changes in EEG motor activity after tSMS have not been tested so far, thus the hypothesis for the present study needed to be in the context of previous studies that have shown a relationship between the EEG beta band and an impairment of motor performance. The rationale for this hypothesis is further explained in *Methodology* (*Chapter 6 – Section 6.2*).

10.2 Methods

10.2.1 Participants

Twenty-six healthy participants (12 males, 22 right-handed) between the ages of 18 and 35 were enrolled in the study were each participant completed two identical visits (intervisit interval range 1–70 days; median = 10.5 days) of real and sham tSMS stimulation during 15 minutes over the dominant hemisphere. All participants underwent equivalent testing: (1) During the first visit, a structured medical history review (see appendix A) and handedness determination were performed. Handedness was determined by revised Edinburgh Handedness Inventory (Oldfield, 1971) (appendix B). TMS and tSMS safety questionnaires were reviewed for all participants at the beginning and end of each visit to screen for possible contraindications (see appendices C and D) and side effects (appendix E). The TMS safety screening form was based on the safety guidelines for TMS by The Safety of TMS Consensus Group (Rossi et al., 2009), for more information read *Chapter 2 – Section 2.6 Safety* of the present thesis. The tSMS safety form was an adaptation from a standard MRI safety questionnaire. The form that was used during the experiments can be found as appendix D. (2) At the beginning of both the real and the sham visits we acquired baseline EEG recordings followed by TMS cortical reactivity and excitability assessments. After the real/sham intervention recordings were repeated in the same order.

In *State of the Art and Methodology* of the present thesis, we stated and reviewed the effects and implications of pulse waveform and current direction on the TMS-brain interaction. After the initial screening and based on these implications, participants were randomly assigned to one of three groups depending on the TMS pulse characteristics for cortical reactivity assessments: ten subjects received $mono_{PA}$, nine received $mono_{AP}$ and seven subjects received $bi_{AP\text{-}PA}$ stimulation. Current directions are referred as the main direction in the motor cortex. Three of the twenty-six enrolled participants were excluded from all data analyses. One participant

(assigned to $mono_{PA}$ group) was excluded because of a past episode of traumatic brain injury with probable loss of consciousness. The other two participants (assigned to $mono_{AP}$ group) could not be included in the analyses of study after the RMT determination because stimulation at suprathreshold intensities (120% of RMT) was not possible due to RMT's greater than 83% of MSO. Therefore, the analyses were performed including 9 participants that underwent $mono_{PA}$ TMS stimulation and 7 $mono_{AP}$ and bi_{AP-PA}, respectively. None of participants that were included in the study had history of medical disease or any contraindication to either TMS or tSMS.

Participants were comfortably seated with their arms rested in a natural 90º angle on a table in front of them. During the recordings and the stimulation, the participants were instructed to remain quiet with their muscles relaxed. Participants were also monitored for drowsiness and asked to keep their eyes open throughout the experiment, unless otherwise specified during EEG recordings.

10.2.2 Transcranial Static Magnetic Stimulation

During each visit, the participants were exposed to either real tSMS or sham intervention. Each participant underwent the real and sham interventions and the order of the real and sham visits was randomly assigned to each participant before the first visit. The real tSMS consisted on a cylindrical neodymium magnet (3.8 cm diameter x 3.8 cm height) (NdFeB; 45 MGOe; megagauss-oersteds, nominal strength 65 kg ≈ 0.5 tesla-T (Model DX8X8 K&J Magnetics, US) with south field polarity (for an up-to-date review of the previous literature and a rationale for the setup, as well as the specific characteristics of the magnetic field, see *Chapters 3 and Chapter 5*, respectively). A non-magnetic metal replica of identical appearance, size and weight was used for sham tSMS. Both interventions had a duration of 15 minutes and were performed over the FDI representation on primary motor cortex of the dominant hemisphere. The FDI cortical representation was previously identified by TMS as the hotspot. Both the magnet and its replica

were held in place with identical elastic bands under the investigators' monitoring. At the end of the second visit, participants were formally asked if they could determine which visit was real and which sham. Only 5 out of the 23 participants (22%) guessed correctly for real/sham visits.

10.2.3 Electromyography

Surface EMG activity was recorded from the dominant hand's FDI using a PowerLab 4/25T data acquisition device and Scope software (ADInstruments, Colorado Springs, CO, USA). Electrodes were placed as described in *Chapter 6 - Section 6.1* using a standard belly-tendon montage over FDI with the ground electrode over the ipsilateral ulnar styloid process. EMG data were digitized at 1 kHz for 250 ms following each stimulus trigger and amplified with a range of ±10 mV (band-pass filter 0.3–1000 Hz). Triggered epochs were acquired for single and paired-pulse measures, while live EMG was recorded and monitored throughout the protocol for the silent period trials to provide feedback for continuous muscle contraction. MEP peak-to-peak amplitudes (mV) of the non-rectified signal for single- and paired-pulse protocols and silent period duration (ms) were measured for individual traces.

10.2.4 Transcranial Magnetic Stimulation

Neuromuscular assessments were performed with neuronavigated TMS using a MagPro X100 device with a hand-held Cool-B65 figure-of-eight coil (outer diameter 75mm) placed over the primary motor cortex in the dominant hemisphere. As argued in *Chapter 4*, the handle of the coil was pointing backwards and at an angle of 45º (MagVenture A/S, Denmark), as this is the optimal coil orientation for motor cortex. MagPro devices are capable of changing between waveforms and current directions without repositioning the coil on the scalp. To assure consistent

targeting throughout the experiment, we used a brain MRI template with a Brainsight TMS neuronavigation system (Rogue Research, Inc., Montreal, QC, Canada) and a Polaris infrared-optical tracking system (Northern Digital Inc., Waterloo, ON, Canada).

Regardless of the waveforms and current directions that were used, the real and sham visits began with the assessment of the motor hotspot. For the hotspot search we used the 10/20 EEG system approach and C3 or C4 as initial references, as previously described in *Methodology* (*Chapter 4 – Section 4.1*). The hotspot, once found, was marked in the template MRI and designated thereafter as the neuronavigation target for the remaining of the visit. The hotspot was researched at the beginning of the second visit with the same methodology. RMT was determined in each visit following hotspot assessment. RMT was defined following the International Federation of Clinical Neurophysiology guidelines (Rossi et al., 2009; Rossini et al., 2015) as the lowest intensity that elicits a MEP of at least 50μV in at least 50% of the trials.

Once RMT was determined, the battery of standard TMS neurophysiological measures of cortical reactivity and excitability were acquired: baseline cortico-motor reactivity; contralateral cSP; and three common paired-pulse protocols interleaved in a pseudorandom sequence. After the 15 minutes of tSMS or sham intervention all the TMS neurophysiological measures, but hotspot search and RMT determination, were repeated in the same order. For each TMS measure, individual data points > 2.5 SD from the mean were excluded from calculation and analysis.

Baseline cortico-motor reactivity was assessed by the average of peak-to-peak amplitude of 40 unconditioned TMS pulses at 120% of RMT. The 40 post-intervention unconditioned MEPs were expressed as a percentage of change from pre-intervention mean MEP amplitude and divided in 4 groups. Each group consisted on the average of 10 consecutive trials. The first group refers to the 10 MEPs acquired just after the real or sham tSMS, and the last group or fourth refers to those acquired around 10 minutes after the intervention. This measure of cortico-motor reactivity will be hereafter referred to as *MEP amplitude*.

The cSP was assessed with 10 single pulses delivered at 120% of RMT during isometric contraction of the FDI at about 25% of the participant's total strength (participants could rest for few seconds between pulses and had constant visual feedback of their performance with the live EMG). The cSP was measured from the onset of the MEP to the resumption of pre-TMS EMG activity (Orth & Rothwell, 2004), and averaged across all 10 trials either pre- or post-intervention.

Paired-pulse protocols included SICI, LICI and ICF using standard parameters (Valls-Solé et al., 1992; Kujirai et al., 1993). SICI consisted of a CP at 80% of RMT, a TP at 120% of RMT and an ISI of 3ms. In LICI, CP and TP were 120% of RMT separated by an ISI of 100ms. ICF consisted of a CS at 80% of RMT, a TP at 120% of RMT and an ISI of 12ms. For further explanations on the standard parameters and the physiological implications see *Chapter 4 – Section 4.3*. Stimulation consisted of 40 individual trials per protocol (for a total of 120 trials), administered in a pseudorandom, interleaved order to reduce blocking effects and with pseudorandomized inter-trial interval (4-6 seconds) to minimize expectation and avoid hysteresis of previous trials. The amplitude of the 40 conditioned MEP for each protocol was expressed as a percentage of unconditioned MEP amplitude and divided in 4 groups of 10 trials (the groups represent the same as described above for MEP amplitude). The post-tSMS percentage of change from pre-intervention was then calculated. Paired-pulse change calculation will be referred to as SICI, LICI or ICF depending on the paired-pulse protocol.

10.2.5 Electroencephalography

Possible changes in neural oscillations after tSMS were assessed analyzing the resting state EEG (rs-EEG) of all right-handed participants (20 total, 8 males). Rs-EEG was recorded using a 32-channel EEG system (BrainVision, BrainProducts, GmbH) with a recording sampling rate of 5000 Hz. Rs-EEG was acquired using the International 10-20 electrode positioning system (***Figure 10.1***) during 2-minutes of eyes-open (EO) and 2-minutes of eyes closed (EC) consecutive

recordings at the beginning of the visit (right before the TMS measures were recorded) and during the 5 last minutes of intervention. The second acquisition was performed during the last 4 minutes of stimulation to ensure a time-window effect for the TMS measures as well. Previous studies have shown that the effects of tSMS on corticomotor reactivity last for about 10 min after the exposure to the SMFs, therefore we expected the EEG effects to be fully present at the time the post-recording started. Ground and reference electrodes were placed in the center of the forehead (AFz) and midline (PCz) respectively, and impedances were kept below 5 kΩ. The spot chosen for the intervention partially overlapped with the position of C3 electrode and therefore this electrode was excluded from recoding in order to reduce the cortex-to-tSMS distance. Electrooculography electrodes (EOG) were placed below and at the outer canthi of one eye to identify vertical and horizontal eye movements.

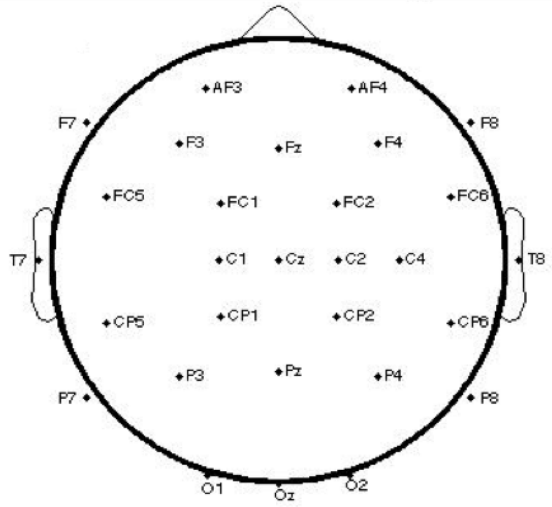

Figure 10.1. Electroencephalography channel positions.

10.2.5.1 Electroencephalography preprocessing

Rs-EEG Pre- and post-intervention data of EO or EC recordings were preprocessed separately and offline using custom scripts in Matlab (version 2012b, Mathworks, USA) and the EEGLab toolbox (Delorme & Makeig, 2004). The first step consisted on filtering the pre- and post-intervention files applying a band-pass filter (high-pass of 1 Hz and low-pass of 100 Hz) and a notch filter (55-65 Hz) for power line noise, both using a zero-phase second-order Butterworth filter. Recordings were then down-sampled to 1024 Hz, continuous data divided into 3-second epochs and pre- and post-intervention files were merged. The merged files were visually inspected and faulty or excessively noisy channels were removed (average number of channels removed 0.10 ±0.50 SD for EO and 0.08 ±0.47 SD for EC). The remaining data was re-referenced to the average of all channels. After re-referencing, the epochs containing excessive artefactual activity were filtered out using a semi-automatically approach where noisy epochs were highlighted. After visual inspection of the files, the noisy epochs were rejected (average number of epochs removed 7.45 ±6.18 SD for EO and 7.18 ±4.60 SD for EC). This led to an average number of epochs of 76.48 (±5.71 SD) for EO and 77.50 (±6.47 SD) for EC per participant being entered for further analysis. Subsequently, independent components analysis (ICA) was performed using the fastICA method (Hyvarinen & Oja, 2000; Rogasch et al., 2014) and components with clear blink, oculomotor, muscle or electrode artifacts were subtracted from the data (average number of rejected components 8.18 ±2.86 SD for EO and 4.95 ±2.12 SD for EC). Previously rejected channels (excluding C3 or C4 depending on the dominant hemisphere and the stimulation site for tSMS) were interpolated using a spherical spline interpolation and the merged files were divided back into pre and post-intervention for subsequent analysis.

10.2.6 Statistical Analyses

10.2.6.1 Electromyography statistical analyses

Stata software version 13.1 (StataCorp, College Station, TX, USA) was used for statistical analyses. Calculation of TMS data for the real and sham interventions for each of the three waveforms/current directions ($mono_{PA}$, $mono_{AP}$, $bi_{AP\text{-}PA}$) included: % change post- to pre-intervention of MEP amplitude; pre- and post- intervention average cSP duration in ms; and % of change of paired-pulse measures (SICI, LICI, and ICF). All analyses were conducted using a two-tailed 95% confidence interval (α=.05).

All data were checked for normality using the Shapiro–Wilk test. MEP amplitude, LICI, SICI and ICF significantly deviated from normality (*p*'s < 0.05), whereas cSP did not (*p*'s > 0.12). Thus, MEP amplitude, LICI, SICI and ICF were transformed as described previously (van Albada & Robinson, 2007).

After normalization of the data, to assess the effect of tSMS on cortical reactivity in both the real and sham visits, we conducted repeated-measures analyses of variance (rm-ANOVAs) with each TMS measure as the dependent variable, the Waveform/current direction, hereafter referred to as *Waveform* ($mono_{PA}$, $mono_{AP}$, or $bi_{AP\text{-}PA}$), as a between-subject variable with nested effects, and *Intervention* (real or sham tSMS) and *Time* (groups of 10 consecutive trials) as longitudinal within-subject variables. Follow-up Tukey's HSD tests were used to conduct pairwise comparisons of the effects of the interventions for each different *Waveform*. Planned contrast analyses were used to conduct pairwise comparisons of the effects of the interventions at each time point with different waveforms/current direction. Results were adjusted for multiple comparisons with the false discovery rate (FDR) method.

Finally, we tested possible effects of a long period of muscle relaxation or cumulative effects of the single-pulse TMS on MEP amplitude by conducting an rm-ANOVA where MEP

amplitudes after the sham condition were the dependent variable, the *Waveform* ($mono_{PA}$, $mono_{AP}$, or bi_{AP-PA}) the between-subject variable with nested effects, *Time* (groups of 10 consecutive trials) as the longitudinal within-subject variable and their interaction.

10.2.6.2 Electroencephalography analyses and statistical procedures for power spectral density

Analyses of the effects of tSMS on cortical oscillatory activity were performed in using custom scripts in Matlab (version 2012b, Mathworks, USA), and the EEGLab (Delorme & Makeig, 2004) and Fieldtrip toolboxes (Oostenveld, Fries, Maris, & Schoffelen, 2011). For the analysis of rs-EEG data of pre/post-tSMS recordings, mean power spectral density across epochs was calculated at all electrodes using the *spectopo* function in EEGlab (sampling rate: 1024 samples, window-overlap = 512) to calculate absolute power for each frequency band. The frequency bands included (total power 1-30 Hz, in steps of 0.5 Hz): Delta 1-3.99 Hz, Theta 4-7.99 Hz, Alpha 8-12.99 Hz and Beta 13-30 Hz. Gamma band (>30 Hz) was not included in the analysis given the current concern about a possible influence of muscle activity and ocular movement on high frequencies when using scalp EEG recordings (Whitham et al., 2007, 2008).

Full rs-EEG data was analyzed performing two-tailed cluster-corrected massive permutation tests to identify significant changes in clusters across the electrodes and frequencies (Bullmore et al., 1999; Groppe, Urbach, & Kutas, 2011; Maris & Oostenveld, 2007). The Monte-Carlo method with a cluster correction approach was chosen in order to control the multiple comparisons problem and for the familywise error rate (FWER). In line with Maris & Oostenveld (Maris & Oostenveld, 2007) the calculation of the test statistics was as follows: based on an initial pairwise comparison of all electrodes and frequencies, the uncorrected p-values that reached an alpha of 0.05 were clustered together if there had at least a neighboring frequency and/or electrode that were significant (average number of neighbor electrodes 4.86, min = 2 and max =

7, numbers were rounded up when no integer number). Subsequently, this cluster-building procedure was repeated across 1000 permutations of the data (Monte-Carlo method) where the most extreme t-scores were retrieved and group level statistics were randomly shuffled. The cluster-building was performed separately for positive and negative t-values. All analyses were conducted using a two-tailed 95% confidence interval (α=.05) and positive and negative clusters were considered significant if lower/higher than 97.5% (2.5% alpha per tail, 5% total alpha level).

These cluster-based massive permutation tests were used to investigate between-group differences at specific time points (pre, post or group regardless of time) and effects of time (pre versus post regardless of the group). The same permutation of cluster-based approach was used for within-group tests (effects for the real and sham interventions through time separately). Two analyses were performed to examine the effects of time and group. First, following the aforementioned method, pre-intervention recordings were subtracted from post and then compared between real and sham groups. Given the restrictive nature of this analysis, we performed a second test within the significant cluster from previous massive permutation tests. For this analysis the average rs-EEG power for a cluster was calculated for each subject as the mean of all significant frequency bins and electrodes in both the real and sham groups for pre and post interventions time points (within-cluster averaged rs-EEG power). First, to elucidate the effects of the intervention for a particular significant cluster we performed a follow-up mixed-effects linear regression. The model included the pre-to-post change of cluster-average rs-EEG power as the dependent variable, *Subject* as a random effect, *Waveform* as a between-subjects factor, *Intervention* as within-subject factor, and *Waveform* x *Intervention* interaction.

Subsequently, to investigate possible relationships between changes in rs-EEG and changes in MEP amplitude a second within-cluster mixed-effects linear regression was performed. Using the within-cluster averaged rs-EEG power, the difference in change between real and sham visits for cluster-average rs-EEG power was the dependent variable, difference in MEP amplitude change between real and sham visits was a covariate, *Subject* was a random

effect and *Waveform* was a between-subjects factor. Following this, follow-up simple linear regressions and Pearson's correlations for each Waveform were completed to clarify possible distinctive relationships depending on the waveform. FDR correction was performed for each linear regression.

10.3 Results

10.3.1 Electromyography results

Table 10.1 shows mean (±SD) of the summary statistics for each TMS neurophysiological measure before and after the intervention as well as how much each measure changed due to the real/sham interventions and the direction of that change.

Table 10.1. Transcranial magnetic stimulation neurophysiological measures.

			Pre-Intervention	Post-Intervention	Effects
MEP amplitude	monoPA	Real	1.4 ± 0.9	1.1 ± 0.9	▼ 24.4%
		Sham	1.2 ± 0.6	1.6 ± 1.5	▲ 41.3%
	monoAP	Real	2.2 ± 1.6	2.5 ± 1.3	▲ 14.7%
		Sham	2.1 ± 1.3	2.2 ± 1.5	▲ 5%
	BiAP-PA	Real	1.8 ± 0.5	2.1 ± 0.7	▲ 20%
		Sham	1.5 ± 0.9	2.2 ± 0.9	▲ 16.5%
cSP	monoPA	Real	119.4 ± 32.5	126.4 ± 25.7	▲ 5.9 %
		Sham	126.2 ± 31.1	136.4 ± 27.6	▲ 8.1 %
	monoAP	Real	127.1 ± 23.3	143.5 ± 50.8	▲ 12.9 %
		Sham	133.4 ± 36.8	135.5 ± 27.5	▲ 1.5 %
	BiAP-PA	Real	139.1 ± 26.3	147.1 ± 18.3	▲ 5.8 %
		Sham	138.7 ± 28.1	148.5 ± 20.0	▲ 7.1 %
% Δ LICI	monoPA	Real	-0.8 ± 0.1	-0.9 ± 0.1	▲ 3.3 %
		Sham	-0.9 ± 0.1	-0.8 ± 0.1	▼ 0.8 %
	monoAP	Real	-0.8 ± 0.4	-0.8 ± 0.4	▲ 1.6 %
		Sham	-0.9 ± 0.2	-0.9 ± 0.2	▼ 0.6 %
	BiAP-PA	Real	-0.9 ± 0.2	-0.9 ± 0.1	▲ 6.5%
		Sham	-0.9 ± 0.2	-0.9 ± 0.2	▲ 1.5 %
% Δ SICI	monoPA	Real	-0.7 ± 0.3	-0.7 ± 0.2	▲ 7.4 %
		Sham	-0.7 ± 0.2	-0.6 ± 0.2	▼ 6.1 %
	monoAP	Real	-0.7 ± 0.3	-0.5 ± 0.7	▼ 31.8 %
		Sham	-0.6 ± 0.4	-0.6 ± 0.5	▼ 0.5 %
	BiAP-PA	Real	-0.5 ± 0.4	-0.4 ± 0.4	▼ 22.3 %
		Sham	-0.6 ± 0.3	-0.4 ± 0.6	▼ 38.7 %
% Δ ICF	monoPA	Real	0.04 ± 0.4	-0.1 ± 0.5	▼ 286.9 %
		Sham	0.2 ± 0.4	0.4 ± 0.7	▲ 145.3 %
	monoAP	Real	0.6 ± 1.0	1.1 ± 1.9	▲ 104.9 %
		Sham	0.3 ± 0.6	0.3 ± 0.6	▼ 11.6 %
	BiAP-PA	Real	0.8 ± 0.7	1.1 ± 1.1	▲ 29.4 %
		Sham	0.6 ± 0.5	1.1 ± 0.8	▲ 87.6 %

Table 10.1 shows mean (±SD) of the different TMS measures performed with the three waveforms and current directions for real and sham interventions. The black arrows indicate the % of increase (▲) or decrease (▼) in mean MEP amplitude (mV) or cSP duration (ms). For paired-pulse protocols the black arrows reflect the % of increase (▲) or decrease (▼) in mean inhibition (LICI, SICI) or facilitation (ICF). *Abbreviations*: % **Δ**, percentage of change from baseline; Bi_{AP-PA}, biphasic anterior-posterior—posterior-anterior; cSP, cortical silent period (ms); ICF, intracortical facilitation, MEP, motor evoked potential (mV); $mono_{AP}$, monophasic anterior-posterior; $mono_{PA}$, monophasic posterior-anterior; LICI, long-interval intracortical inhibition; SICI, short-interval intracortical inhibition.

MEP amplitude inhibition after real tSMS was only observed when TMS was performed with $mono_{PA}$ (inhibition of 24.4 %)(***Table 10.1***). The rm-ANOVA analysis for the MEP amplitude showed a significant effect of *Waveform* ($p < 0.001$) and of *Waveform* x *Intervention* interaction ($p < 0.001$). No significant effects of *Intervention* or *Time* alone or for the rest of the interactions (i.e. *Waveform* x *Time*, *Intervention* x *Time* or *Waveform* x *Time* x *Intervention*) were observed (all *p*'s > 0.05). Post Hoc Tukey's HSD and planned contrast analyses, of *Waveform* and the *Waveform* x *Intervention* interaction respectively, showed a significant difference between $mono_{PA}$ with both $mono_{AP}$ and bi_{AP-PA} waveforms (*p*'s < 0.05). $Mono_{PA}$ was the only waveform that significantly inhibited after the real intervention ($p < 0.001$) whereas $mono_{AP}$ MEP amplitudes were significantly facilitated ($p = 0.02$) (***Figure 10.2***). Furthermore, the inhibitory effects of real tSMS when evaluated by $mono_{PA}$ were significantly greater than sham at Post-T3 and Post-T4 (i.e. pulses 21 to 30 and 31 to 40, respectively) (both *p*'s < 0.02) (***Figure 10.3***). Follow-up contrast analysis also showed an increase of mean MEP amplitude at real Post-T3 compared to sham condition that did not survive FDR correction ($p = 0.18$)**.** Bi_{AP-PA} waveform did not differ significantly from $mono_{AP}$ and the slightly facilitatory effects of the tSMS captured by Bi_{AP-PA} did not reach significance.

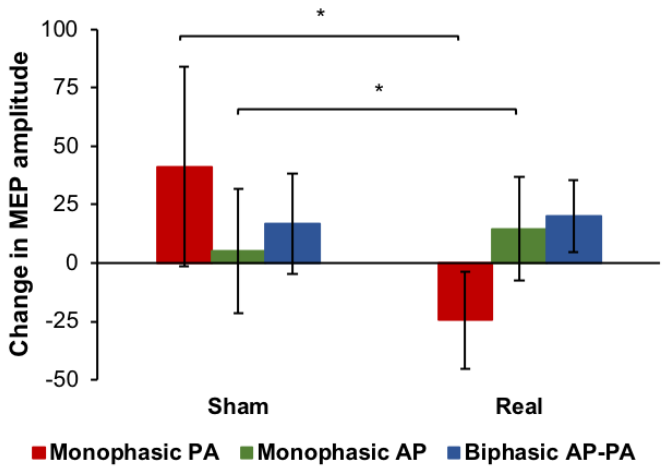

Figure 10.2. Change in motor evoked potential (MEP) amplitude from pre- to post-intervention for each waveform and current direction.

The figure shows the mean ± SE for the percentage of change in MEP amplitude. The negative values represent a decrease in MEP amplitude compared to pre-intervention. *Abbreviations*: AP, anterior-posterior; AP-PA, anterior-posterior—posterior-anterior; PA, posterior-anterior. * $p < 0.05$.

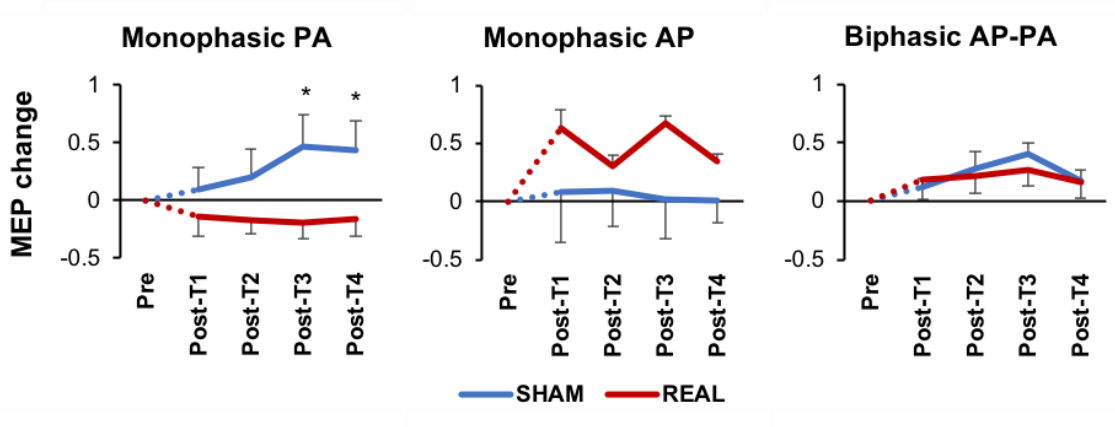

Figure 10.3. Change in motor evoked potential (MEP) amplitude trough time.

The figure shows the mean ±SE of the change in MEP amplitude from baseline through time for each waveform and current direction. The sham intervention is represented in blue and the real in red. The dashed lines depict the transition time from pre-intervention (Pre) to post-intervention (PostT1-T4) where the intervention took place. Only $mono_{PA}$ showed a significant difference in the change of MEP amplitudes between real and sham over time (* *p*'s < 0.02). *Abbreviations*: AP, anterior-posterior; AP-PA, anterior-posterior—posterior-anterior; PA, posterior-anterior.

When only sham MEP amplitude was included in the model searching for possible effects of a long period of muscle relaxation or hysteresis of single-pulse TMS, no significant differences were found in the amplitude for *Waveform*, *Time* or their interaction (all *p*'s > 0.3).

All the *Waveforms* lengthened the duration of cSP regardless of the *Intervention* (***Table 10.1*** and ***Figure 10.4***). Accordingly, the rm-ANOVA for cSP showed a significant effect of *Waveform* ($p < 0.01$) and *Time* ($p = 0.02$) but no significant differences were found either for the *Intervention* or any of the interactions. Post hoc Tukey's HSD for *Waveform* found that Bi_{AP-PA} was significantly different than both monophasic waveforms ($p < 0.05$) and $mono_{PA}$ inhibited more after the real intervention ($p = 0.1$), however none of the rest of follow-up analyses showed different effects in relation to the *Intervention*.

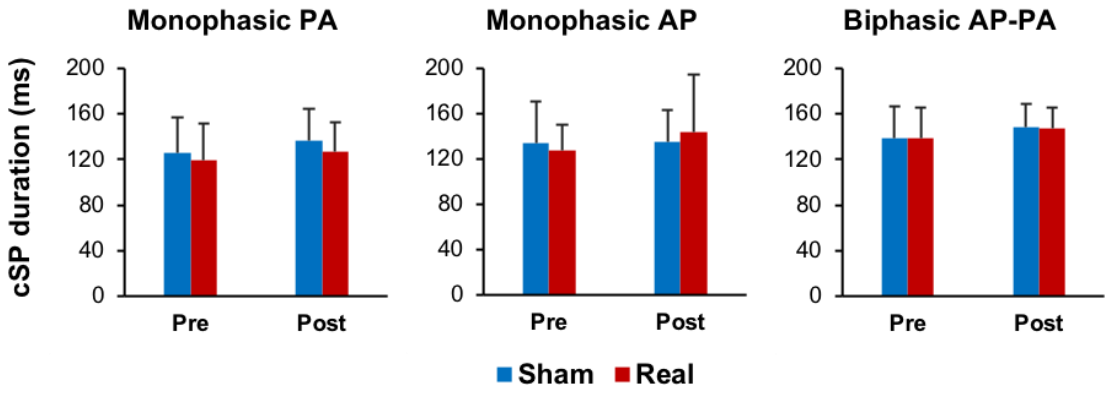

Figure 10.4. Cortical silent period (cSP) durations in pre- and post-interventions.
The figure shows the mean ± SE of cSP duration in ms for pre- and post-intervention for the different waveforms and current directions. The sham intervention is in blue and the real in red. *Abbreviations*: AP, anterior-posterior; AP-PA, anterior-posterior—posterior-anterior; PA, posterior-anterior.

For the change in LICI, all waveforms were able to elicit greater inhibition after the real intervention (***Table 10.1***), furthermore both *Waveform* and *Waveform* x *Intervention* interaction were significant (*p*'s < 0.001). Post Hoc Tukey's HSD showed that all waveforms were significantly different form each other (*p*'s <0.05) but only the monophasic ($mono_{PA}$ and $mono_{AP}$) were able to

significantly inhibit after real tSMS (*p*'s < 0.01) (***Figures 10.5*** and ***10.6***). The effects of *Time* and its interactions did not reach significance.

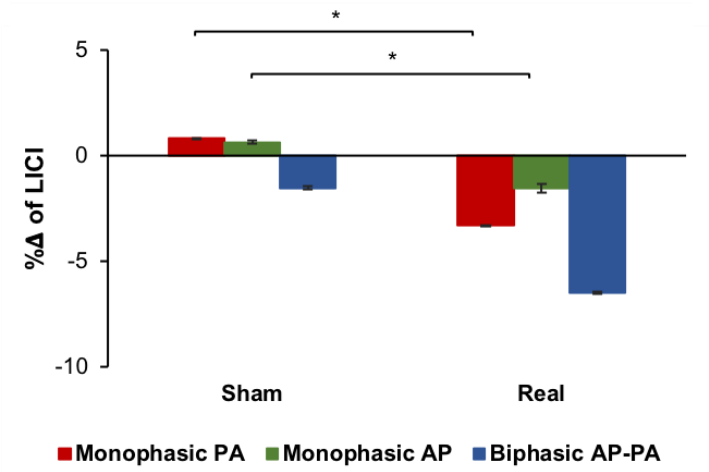

Figure 10.5. Change in long-interval intracortical inhibition (LICI) from pre- to post-intervention for each waveform and current direction.
The figure shows mean ±SE of the percentage of change of LICI. The negative values represent more inhibition compared to pre-intervention. *Abbreviations*: AP, anterior-posterior; AP-PA, anterior-posterior—posterior-anterior; PA, posterior-anterior. * *p*'s < 0.01

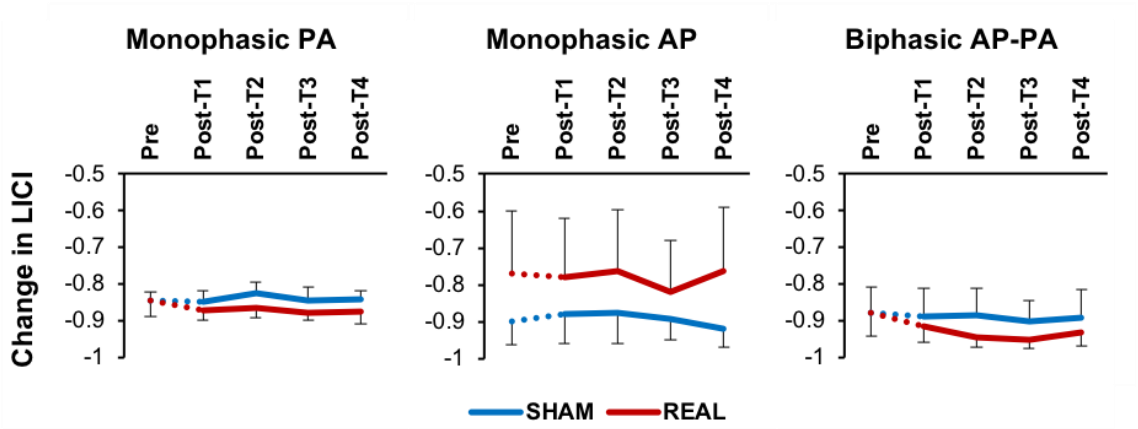

Figure 10.6. Change in long-interval intracortical inhibition (LICI) through time.
The figure shows the mean ±SE of the change in LICI from pre-intervention baseline through time for each waveform and current direction. The sham intervention is represented in blue and the real in red. The dashed lines depict the transition time from pre-intervention (Pre) to post-intervention (PostT1-T4) where the intervention took place. *Abbreviations*: AP, anterior-posterior; AP-PA, anterior-posterior—posterior-anterior; PA, posterior-anterior.

When performing SICI, only $mono_{PA}$ waveform captured an increase in inhibition after real tSMS (***Table 10.1***). The rm-ANOVA yielded significant *Waveform* and *Waveform* x *Intervention* interaction effects (both *p*'s < 0.001). Post Hoc Tukey's HSD analyses showed that Bi_{AP-PA} significantly differed from the other two waveforms ($p < 0.05$). Planned contrast tests revealed a significant increase in inhibition after real tSMS for $mono_{PA}$ and when compared to sham ($p = 0.005$). Bi_{AP-PA} and $mono_{AP}$, both showed a decrease in inhibition after both interventions. Nevertheless Bi_{AP-PA} showed a relative increase in inhibition after real when compared to sham ($p = 0.005$) and $mono_{AP}$ a significant decrease inhibition (relative facilitation) after real intervention ($p < 0.001$) (***Figure 10.7***). None of the specific time points post-intervention survive FDR correction (***Figure 10.8***).

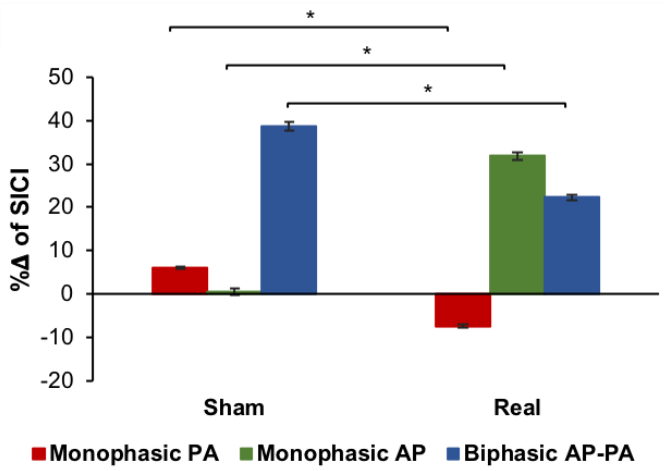

Figure 10.7. Change in short-interval intracortical inhibition (SICI) from pre- to post-intervention for each waveform and current direction.
The figure shows mean ±SE of the percentage of change of SICI. The negative values represent more inhibition compared to pre-intervention. *Abbreviations*: AP, anterior-posterior; AP-PA, anterior-posterior—posterior-anterior; PA, posterior-anterior. * *p*'s < 0.05.

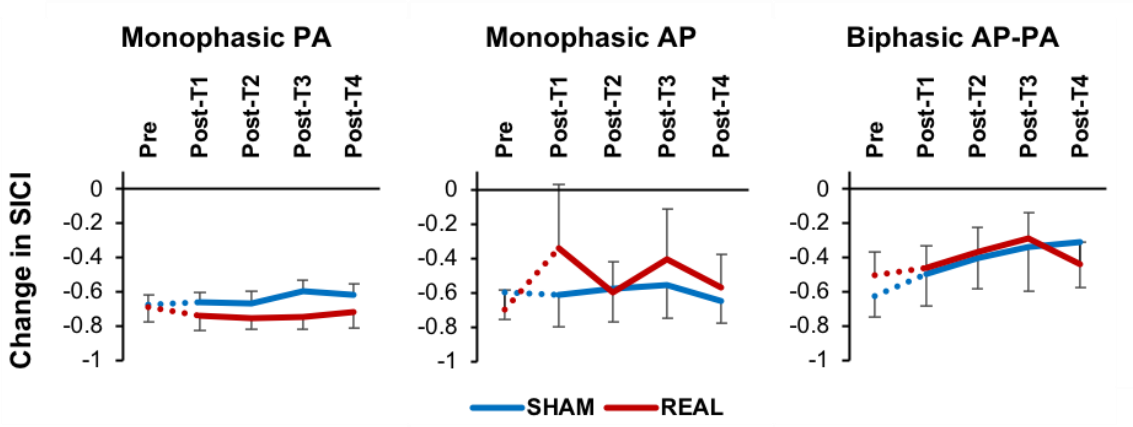

Figure 10.8. Change in short-interval intracortical inhibition (SICI) through time.
The figure shows the mean ±SE of the change in SICI from pre-intervention baseline through time for each waveform and current direction. The sham intervention is represented in blue and the real in red. The dashed lines depict the transition time from pre-intervention (Pre) to post-intervention (PostT1-T4) where the intervention took place. *Abbreviations*: AP, anterior-posterior; AP-PA, anterior-posterior—posterior-anterior; PA, posterior-anterior.

Finally, only one of the paired-pulse TMS protocols performed in this experiment reflects the facilitatory mechanisms of the motor cortex. For ICF protocol, the only waveform that was able to capture an overall decrease in facilitation after real tSMS was $mono_{PA}$ while both $mono_{AP}$ and $bi_{AP\text{-}PA}$ facilitated to some extent (***Table 10.1*** and ***Figure 10.9***). However, due to the high variance of the sample the rm-ANOVA yielded no significant effects of Waveform, Intervention, Time or their interactions (p's > 0.15) (***Figure 10.10***).

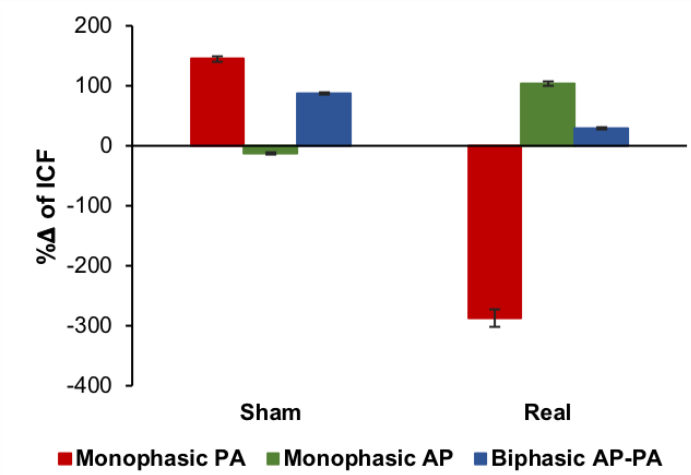

Figure 10.9. Change in intracortical facilitation (ICF) from pre- to post-intervention for each waveform and current direction.

The figure shows mean ±SE of the percentage of change of ICF. The negative values represent less facilitation compared to pre-intervention. *Abbreviations*: AP, anterior-posterior; AP-PA, anterior-posterior—posterior-anterior; PA, posterior-anterior.

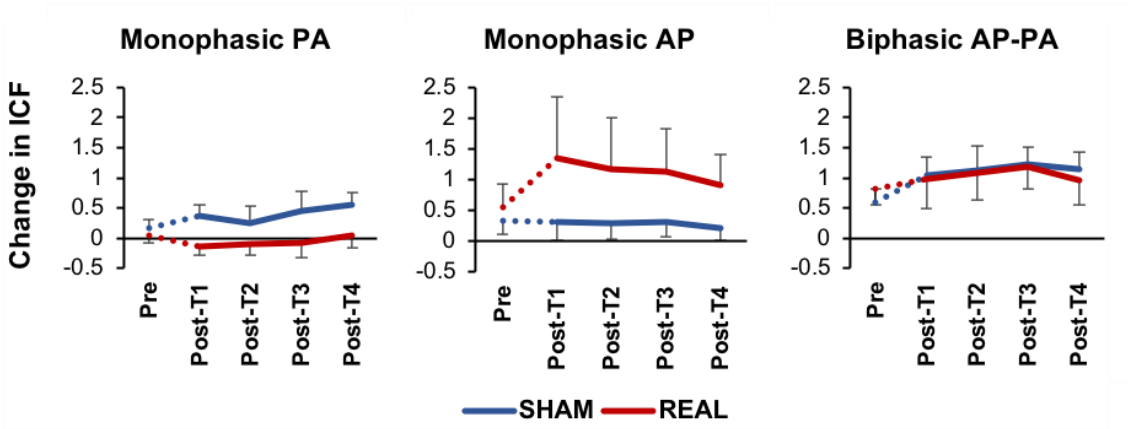

Figure 10.10. Change in intracortical facilitation (ICF) through time.

The figure shows the mean ±SE of the change in ICF from pre-intervention baseline through time for each waveform and current direction. The sham intervention is represented in blue and the real in red. The dashed lines depict the transition time from pre-intervention (Pre) to post-intervention (PostT1-T4) where the intervention took place. *Abbreviations*: AP, anterior-posterior; AP-PA, anterior-posterior—posterior-anterior; PA, posterior-anterior.

10.3.2 Electroencephalography results

For the EO condition, the two-tailed cluster-corrected massive permutation tests yielded a significant difference in pre- versus post-intervention recordings when both groups were shuffled together, although no differences were found in the between-groups analysis at different time points (*p*'s > 0.19). The pre- versus post-intervention analysis revealed a significant increase in the alpha (p = 0.005) and beta bands (p = 0.006). ***Figure 10.11*** shows the cluster-corrected significant electrodes and frequency bins for both significant clusters and a representative topography for each of them.

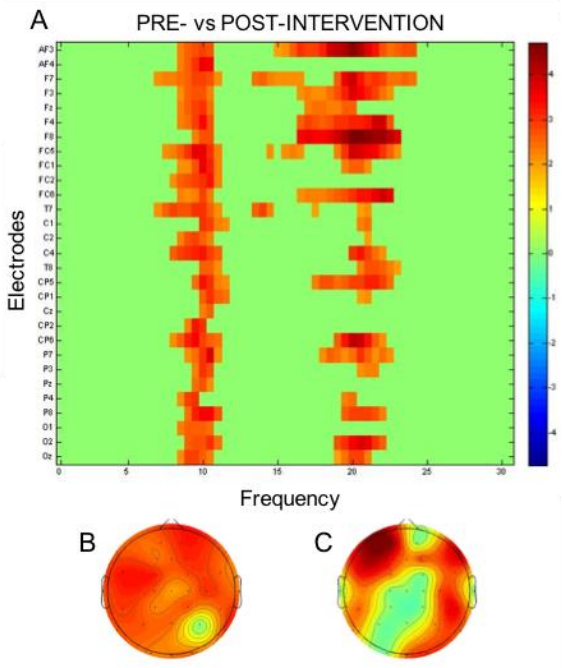

Figure 10.11. Whole-brain analysis of absolute power for Time effects of tSMS. **A**. Cluster-corrected *T*-values associated with an increment of alpha and beta bands comparing rs-EEG pre- to post-intervention in both groups (real and sham tSMS) across all electrodes (y-axis) and frequencies (x-axis). **B-C.** Characteristic topographic representations of the *t*-values associated with the rise in alpha (10Hz; t = 390, p = 0.006) (**B**) and beta (20Hz; t = 530, p = 0.005) (**C**) bands.

Furthermore, for the within-group comparisons for real and sham interventions separately showed a significant increase in the wide range of beta band (16-30 Hz) ($p < 0.004$) in bilateral fronto-central electrodes with a left hemisphere predominance after real tSMS with no significant changes after the sham intervention. ***Figure 10.12*** shows the increase in beta after the real intervention and a representative topography for the significant cluster, and the lack of rs-EEG effects of sham.

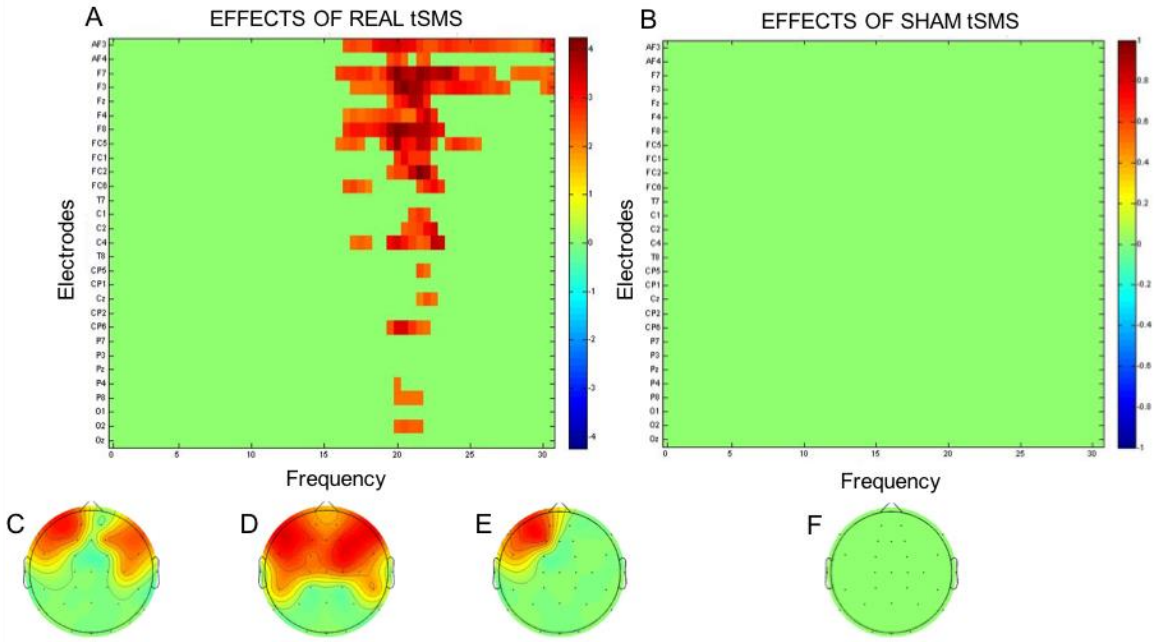

Figure 10.12. Whole-brain analysis of absolute power for each pairwise comparison.
A-B. Cluster-corrected *T*-ratios between the pre- and the post-intervention for real and sham tSMS associated with an increment of beta band (**A**) after the real intervention but no significant effects after the sham (**B**) across all electrodes (y-axis) and frequencies (x-axis). **C-F.** Characteristic topographic representations of the *t*-values associated with the rise in fronto-central beta band (17, 22 and 25 Hz; $t = 545$, $p = 0.004$) (**C-E**) after the real tSMS and no significant effects after sham (**F**).

To further investigate the effects of the real versus sham tSMS when rs-EEG was recorded with EO, we performed two analyses. First, a massive permutation cluster-corrected test with all electrodes and frequencies included that led to no significant differences and a second analysis where the specific significant electrodes and frequencies from the within-group cluster were averaged together and compared. This within-cluster mixed-effects linear regression that showed an effect of the *Intervention* (p = 0.03) with no *Waveform* or the *Waveform* x *Intervention* interaction (p > 0.11) effects.

No significant effects of tSMS were found in the recordings performed with EC.

Possible relationships between the changes in EO rs-EEG and the changes in MEP amplitude were also investigated. The mixed-effects linear regression yielded significant effects of waveform (p = 0.002) with mild effects of the change in MEP amplitude (p = 0.061). Follow-up linear regressions for each waveform revealed a significant relationship between rs-EEG and MEP amplitude changes when $mono_{PA}$ TMS (p = 0.036) was utilized but not when $mono_{AP}$ or bi_{AP-PA} (p's > 0.26) were the waveforms of choice. This relationship was additionally investigated with Pearson's correlations. The results of the correlations are depicted in ***Figure 10.13***.

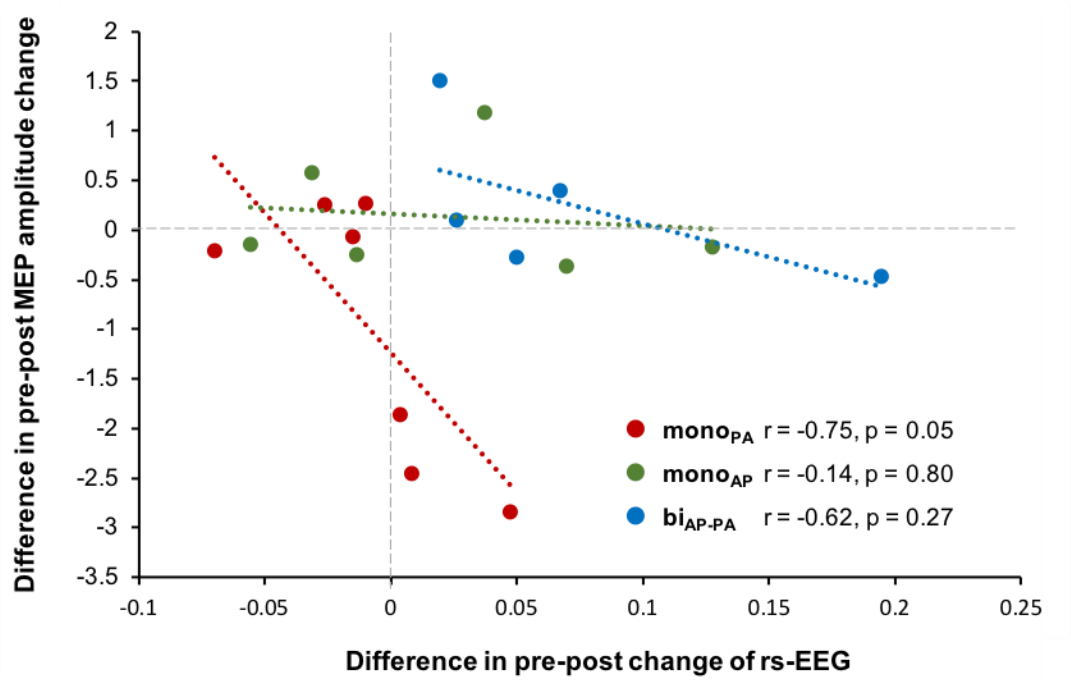

Figure 10.13. Relationships between the differences in MEP amplitude (y-axis) and beta band (x-axis) changes between real and sham tSMS for each waveform.
After tSMS the mono$_{PA}$ pulses revealed an inhibition of the MEP amplitudes that is negatively correlated with the increase in rs-EEG beta band. A moderate negative correlation between the decrease in MEP amplitude and the increase in beta band was also found for bi$_{AP-PA}$ and no relationship between the MEP and EEG changes was achieved for mono$_{AP}$.

10.4 Discussion

The present study investigated the effects of real and sham tSMS on motor cortex excitability, cortical balance of inhibition and facilitation, and brain oscillatory spontaneous activity. Both, the cortical excitability (expressed in terms of MEP amplitude) and the intracortical balance of inhibitory and facilitatory processes (measured by cSP, LICI, SICI and ICF) were evaluated with different TMS waveforms and current directions. After the real intervention, the MEP amplitude and the paired-pulse protocols LICI and SICI showed an increase in intracortical inhibition, while cSP tended to have a longer duration and ICF tended towards less facilitation of

the conditioned MEPs. These inhibitory results, however, appeared only when the consequences of tSMS where explored with $mono_{PA}$. An overall facilitation of MEP amplitude and less inhibition after SICI were revealed with $mono_{AP}$, however LICI induced more inhibition and no change was observed in cSP or ICF. Therefore, there was an increase in cortical excitability measured by single-pulse MEPs but no clear tilt in the balance was observed when TMS was performed with $mono_{AP}$. No changes in excitability or intracortical balance were significant for bi_{AP-PA} waveform. Associated with the real tSMS there was as well an increase in the brain spontaneous oscillations (rs-EEG) in particular an increase in beta band power in the bilateral front-central region with a left hemisphere predominance. This increase was observed after the real-intervention visit regardless of the TMS waveform used for the evaluation of corticospinal effects. Furthermore, this increase in beta power, although present in the whole real group, was highly correlated with the reduction in MEP amplitude seen after $mono_{PA}$, mildly related to responses of bi_{AP-PA} with no relation to $mono_{AP}$ excitability changes.

In order to understand the present EMG findings, the best framework available nowadays is the theoretical canonical cortical model proposed by several authors in the past (Di Lazzaro, Oliviero, Mazzone, et al., 2001; Di Lazzaro, Oliviero, Saturno, et al., 2001; Di Lazzaro et al., 2006; Di Lazzaro, Ziemann, & Lemon, 2008; Di Lazzaro et al., 2011; Di Lazzaro & Ziemann, 2013; Di Lazzaro et al., 2017), this model and the effects of waveform and current direction on motor cortex responses are further explained in *Chapter 2 – Section 2.3* of the present thesis.

Previous studies on the effects of tSMS on the motor cortex excitability have found a decrease in MEP amplitude of about 25% after tSMS, that was first shown by Oliviero et al. (Oliviero et al., 2011) and posteriorly replicated by Silbert et al. (Silbert et al., 2013), both groups used $mono_{PA}$ for their TMS procedures. Our results, in line with these prior reports, showed that the effects of the real tSMS yielded an average MEP amplitude decrease of 24.4% when the excitability of motor cortex was explored using the same type of waveform.

Moreover, we also explored the course of these inhibitory effects through time and in relation to the responses to sham intervention. Relatively to sham, the inhibitory effects after the real tSMS were enlarged, showing a decrease up to a 60% when real and sham were directly compared. This difference between real and sham cortical excitability was not only due to a decrease of MEP amplitude after real but also because there was a tendency towards facilitation of the MEPs after sham. In contrast to the reduction of the MEP amplitude, the increase of sham MEPs was captured by all waveforms and current directions as time went on with no significant difference between them. The increase of the motor responses after sham or no intervention has been previously related to prolonged periods of muscle relaxation (Todd, Butler, Gandevia, & Taylor, 2006) or possible cumulative effects of single-pulse TMS (Pellicciari, Miniussi, Ferrari, Koch, & Bortoletto, 2015). Todd et al. found an increase of about 50% of MEP average duration after 20 minutes of muscle relaxation with no increase in peripheral muscle response (M wave) or spinal cord excitability (F wave). The authors, that used $mono_{PA}$ TMS, argued that this change could be mainly explain by an increase in cortical excitability due to the lack of motor input signals. Their hypothesis is also supported by previous experiments with ischemic or anesthetic nerve blocks (Brasil-Neto, Cohen, Pascual-Leone, et al., 1992; Ziemann, Corwell, & Cohen, 1998) where the cortical excitability raised after a short period of time. On a second study, Pellicciari et al. (Pellicciari et al., 2015) studied the effects of blocks of TMS biphasic single-pulses over time with fixed and random ISIs. The authors found that independently of the pattern of ITIs the MEP amplitude increased over time, and argued that single-pulse TMS may have a modulatory cumulative effect on corticospinal excitability similar to the modulation seen after rTMS.

All of the above suggests that the local SMFs of tSMS not only decrease cortical reactivity to some extent but also prevent a raise in motor cortex excitability in response to either the muscle inactivity or the cumulative facilitatory effects of single-pulse TMS. In our results, this difference between real and sham for $mono_{PA}$ waveform was more evident at times T3 and T4 (about 5-6 minutes after the intervention).

While our results using $mono_{PA}$ TMS are in line with those previously reported (i.e. exploring certain intracortical circuits), it is important to highlight that this decrease in motor cortex excitability was not observed with either $mono_{AP}$ or $bi_{AP\text{-}PA}$. Intriguingly, the effects captured by $mono_{AP}$ were facilitatory showing an average increase of MEP amplitude after the real tSMS. To explain this increase, we refer to the canonical cortical model and the specific neural circuits that $mono_{AP}$ explores. As mentioned above, $mono_{AP}$ evaluates cortico-cortical connections that most probably have regulatory inputs from other cortices or brain structures to contribute to motor control. One possibility may be that the SMFs inhibit those regulatory inputs releasing those cortico-cortical connections that are explored with $mono_{AP}$. This hypothesis should be further tested with future studies perhaps recording from epidural electrodes to test whether there is an increase in the late I-waves amplitude after real tSMS when evaluated by $mono_{AP}$. Besides the results of monophasic waveforms, biphasic competing mechanisms may take place by activating both the cortico-cortical connections (AP component) and the inhibitory intracortical networks in layers II and III (PA component). Congruently, biphasic MEP amplitudes did not show any significant decrease or increase after the real intervention or when compared to sham.

In addition to cortical excitability, the balance between inhibition and facilitation of the motor system was evaluated with paired-pulse TMS measures. Greater inhibition after LICI and SICI was achieved with $mono_{PA}$ waveform, as well as a tendency towards larger cSP durations and less facilitatory cortical responses. Also important, the intracortical balance was shown to remain without clear overall significant alterations when evaluated by $mono_{AP}$ or $bi_{AP\text{-}PA}$ given that both increases and decreases of inhibitory processes were observed when using those waveforms.

The single- and paired-pulse inhibitory protocols that were evaluated in the present experiments included SICI, LICI and cSP. All these protocols translate cortical GABA processes but there are some subtle differences between them. GABA is the primary inhibitory neurotransmitter of the CNS and has two main membrane receptors, $GABA_A$ and $GABA_B$. While

SICI is mediated by $GABA_A$ processes that exert a fast ionotropic inhibition (Cherubini, 2010), LICI and cSP are mediated by $GABA_B$ translating slow metabotropic inhibitory processes (Mott, 2015). In addition, epidural recordings have shown that SICI and LICI produce a reduction in the amplitude of late I-waves with preservation of the I1-wave, whereas in the cSP protocol there is a first facilitatory phase where both the I1-wave and the late I-waves are enhanced with a posterior reduction or inhibitory phase (Di Lazzaro et al., 2017). To sum up, each inhibitory protocol despite their similarities most probably activate distinctive neural circuits through a common inhibitory neurotransmission system with different membrane receptors. However, as already mentioned in *Chapter 7*, the measurements of the epidural volleys have only been performed with $mono_{PA}$ waveform, hence future studies should investigate the epidural responses to other waveforms and current directions as also suggested in this same chapter of the present thesis. This will help to identify possible mechanistic differences between protocols and the tSMS effects when multiple waveforms are used.

Since each inhibitory protocol may translate specific cortical processes, this allows to anticipate subtle different effects of the fields of the tSMS for each one of them and theorize about possible physiological implications. In a previous study, Nojima et al. (Nojima et al., 2015) evaluated the effects of tSMS on SICI, cSP and ICF when explored with $mono_{PA}$ waveform. Comparable to our results, the authors found greater inhibitory effects of SICI and a tendency toward longer cSP durations, suggesting that GABA-mediated inhibitory processes may be involved in the reduction of cortical excitability observed after real tSMS. As previously mentioned, cSP is a complex motor response that involves first facilitatory response followed by an inhibitory phase, and also involves both cortical and spinal processes which may interfere with the effects of tSMS. Therefore, the results of cSP may be more difficult to resolve. However, the authors did not find any reduction in facilitation after ICF, whereas our results suggest a trend towards lower facilitation after real tSMS reflecting an overall cortical inhibition. It is probable that decreasing the variance of the sample (by either increasing the number of pulses, the *n* of the sample or

modifying the technical parameters for a more stable ICF response) this reduction in facilitation may be more obvious. In regards to this last point, it has been suggested that a minimum of 30 pulses is required for a reliable response to ICF protocol (Biabani, Farrell, Zoghi, Egan, & Jaberzadeh, 2018). Thus, it is probable that in future studies more pulses are needed in order to clarify whether facilitatory processes may be also affected by tSMS.

The experiments of the present work testing the cortical balance of inhibition and facilitation have expanded those previously reported by evaluating a third inhibitory protocol, LICI, which is, like cSP, $GABA_B$-mediated and also by exploring the excitability and intracortical balance with different waveforms and current directions. In regards to LICI, our results showed an increase of LICI cortical responses associated to greater inhibition.

In summary, tSMS reduces cortical excitability and tilts that intracortical balance towards inhibition, but these effects can be only evaluated with $mono_{PA}$. Thus, these results suggest that the tSMS may not only affect GABA-mediated processes but also particular neural circuits. In other words, the effects of SMFs may selectively affect specific networks of intracortical interneurons in layers II and III that are evaluated with $mono_{PA}$, while the other neural circuits seem to remain mostly non-inhibited.

Besides the EMG responses, during our experiments we have also recorded the spontaneous brain oscillatory activity before and after the interventions. The exposure to tSMS in the real intervention produced an increase in beta oscillations in bilateral fronto-central regions. Reassuringly, this increase in beta was observed in the group that received real tSMS regardless of the TMS waveform that was used for posterior excitability and cortical balance assessments.

Moreover, the EMG and rs-EEG changes were found to be highly negatively correlated when TMS was performed with $mono_{PA}$ and mildly negatively related when $bi_{AP\text{-}PA}$ was used. No relationship was found for $mono_{AP}$. Reinforcing the idea that tSMS alters specific neural circuits within the motor cortex.

To better understand the relationship between the EMG and EEG results is important to provide a context for the behavioral meaning of beta oscillatory activity in the motor cortex. Beta is a prevalent oscillatory frequency in the motor system and has long been associated with the somatosensory networks, but its significance and behavioral correlates are yet very poorly understood (Jenkinson & Brown, 2011). As stated in *Chapter 6*, different theories have been proposed along the years. The first accepted hypothesis is that beta is a resting rhythm that represents a reliable indicator of the cortical control for movement prevention and initiation (Espenhahn, de Berker, van Wijk, Rossiter, & Ward, 2017; Jenkinson & Brown, 2011). In accordance with this, beta activity is suppressed at the onset and during the movement, and rebounded as soon as the movement stops. Similarly, beta power is enhanced when a movement needs to be ceased or voluntarily suppressed (Pogosyan et al., 2009; Zhang, Chen, Bressler, & Ding, 2008). This relationship is also supported by studies that showed that, in healthy individuals, slowed or worse motor performances are highly associated with an increase in spontaneous (Gilbertson et al., 2005) or entrained (Pogosyan et al., 2009) beta power. Increases of beta power in the motor network are also behind and reflecting a causal relationship to akinetic and dyskinetic symptoms of Parkinson's Disease (Brown, 2007; Little & Brown, 2014). The second theory of a behavioral equivalence suggests that the beta oscillations are not only a resting rhythm but the expression of the efforts of the brain to maintain the status quo or the current motor state (Engel & Fries, 2010).

Therefore, the highly negative correlation between the increase in rs-EEG beta band and the decrease in MEP amplitude observed with $mono_{PA}$ in our experiments, are in line with previous reports on motor behavior and EEG relationship. It is also very probable that the biphasic pulse partially evaluates, with its PA component, the same neural circuits than $mono_{PA}$ and that is reflected in a mild-to-moderate relation between the rs-EEG and biphasic EMG changes. Although, due to the AP component and its possible counterbalancing effect in motor cortex, those changes were not enough to reflect a significant reduction in MEP amplitudes.

Additional rs-EEG analyses investigated the effects of time regardless of the intervention showing two significant clusters. First, a fronto-central increase in beta that was led by the real tSMS group (i.e. no increase of beta was observed in the sham group in the pairwise comparison), and secondarily a raise in global alpha frequency across the entire brain. Despite the efforts of the investigators encouraging the participants to remain awake and focused, this raise in alpha frequency is most likely related to a higher level of drowsiness over time regardless of the intervention. The cluster-plot as well as the topographic representation of the alpha cluster show that the increased frequencies are mainly centered around 8-10Hz and that those frequencies are distributed globally through all electrodes. These features are often interpreted as an indicator of drowsiness or sleepiness.

Finally, no significant results were observed when the recordings where performed with the participants having their eyes closed. Previous studies have suggested that the effects of different brain stimulation techniques depend on specific brain states (Neuling, Rach, & Herrmann, 2013; Thut, Schyns, & Gross, 2011). Therefore, it has been proposed that the neurophysiological recordings should be performed during the same brain state the NIBS technique was applied. Furthermore, specific rhythms are more effectively entrained in a brain state that physiologically favors such particular frequency. Namely, alpha rhythms can be more easily induced in dark conditions or eyes closed, whereas beta promotion would be best in light or with the eyes open (Kanai, Chaieb, Antal, Walsh, & Paulus, 2008). Given that during the exposure to either real or sham tSMS the participants were asked to remain with their eyes open, the main effects were expected to be maximized when the rs-EEG was recorded with the brain in that same condition. As one may expect, the effects of tSMS on the recordings performed with EC in the present experiment did not reach to any significant changes. This is probably due to a change in the brain state (from EO to EC) together with the fact that our main findings were observed in beta band which is best promoted during EO.

10.5 Conclusions

The motor cortex exposure to tSMS changes brain's excitability, the intracortical balance between inhibitory and facilitatory processes, and spontaneous oscillatory activity generating greater cortical inhibition that can be monitored with both EMG responses and EEG recordings. Those effects influence particular neural networks within the motor cortex. Most probably, tSMS changes the excitability of cortical interneurons in layers II and III given that only the TMS waveform $mono_{PA}$ was able to quantify those changes.

Further evidence comes from the recording of spontaneous oscillatory activity, as measured by rs-EEG. Oscillations experienced an increase in a wide range of the beta frequency (16-30 Hz) after the real intervention regardless of the TMS waveform used posteriorly. Moreover, this boost in beta activity was highly correlated with the decrease in MEP amplitude observed with $mono_{PA}$, reinforcing the hypothesis of excitability changes in specific intracortical circuits.

ADDITIONAL CONSIDERATIONS AND GENERAL CONCLUSIONS

11 Additional considerations

Few factors may limit the generalizability of the results of the present thesis. The most prominent limitation common to several experiments is the relatively small sample size in the groups of study. While the sample sizes may seem small, this has not reduced the power to detect significant differences between groups. It should be noted that in the present thesis, (1) we were able to detect significant differences between the groups of the different experiments; (2) our results are in line and have expanded previously reported measures in the literature; (3) in the reliability studies (*Chapters 7 – 9, Reliability studies*), when comparing the young versus the older healthy controls, several of the presented reliability coefficients were quite close between both groups regardless of being obtained from separate cohorts, in different studies and with different TMS stimulators.

Some other limiting factors are referred to the intrinsic design of a particular study or the way the subjects were chosen. In the studies were the outcomes of different waveforms and current directions are compared (i.e. *Chapters 7* and *10*) a within-subject comparison of these parameters would have resulted in more power to detect significant differences in the efficacy and

reliability of TMS measures (*Chapter 7*) and in the effects of tSMS associated with pulse waveform and induced current direction (*Chapter 10*). However, completing those studies using a within-subject design would have required six visits per subject, which may have reduced the feasibility of our study due to attrition. Importantly, a fully within-subject design would have made it difficult to disentangle the reproducibility results from the efficacy comparisons in *Chapter 7*. Finally, in the group of T2DM (*Chapters 8* and *9*), a number of the non-diabetic controls (i.e. Healthy controls) had hemoglobin A1c values indicating possible pre-diabetes. This may have contributed to the decreased reproducibility seen in this cohort. Further, HbA1c values were not available from the AD group, so the influence of impaired glucose metabolism could not be investigated in AD subjects, despite reports of high co-morbidity between AD and T2DM (Hölscher, 2011).

Future studies, apart for including larger cohorts, should investigate possible differences in the response to iTBS between young and older healthy controls.

12 General Conclusions

In the first set of experiments, we aimed to better understand factors that could influence the reliability of different TMS measures. Those experiments included technical or modifiable factors (i.e. TMS pulse waveform and current direction), and physiological or non-modifiable (i.e. age and age-related diseases). Based on the results of the present thesis several general conclusions can be derived:

1. Motor thresholds and MEP latencies remain the gold standard for reproducibility regardless technical or physiological factors. Nevertheless, waveform and current direction influence single-pulse TMS measures such as RMT, latency and cSP.

2. Paired-pulse protocols were performed with waveforms and current directions that have not been typically studied in previous literature (i.e. $mono_{AP}$ and bi_{AP-PA}). Monophasic waveforms achieved greater and more reliable inhibition. On the other hand, facilitatory protocols showed greater and more reliable effects when performed with bi_{AP-PA}.

3. When comparing different populations, higher RMT and LICI reliability coefficients were observed in OHC than in younger controls, and the AD group showed higher post iTBS reliability relative to OHC or DM2. The greater reliability coefficients translate less variability in the measures probably due to greater cortical rigidity.

4. Two factors have been found to contribute to increase the variability of the responses to iTBS: (1) Met-carriers of the BDNF-polymorphism, (2) and days between visit, where intervisit intervals of less than a week probably reflected metaplasticity processes of iTBS.

In the second set of experiments, we investigated the effects of tSMS on cortical excitability, intracortical balance of inhibition and facilitation, and brain spontaneous oscillations (rs-EEG). From these experiments we conclude that:

1. Fifteen-minute tSMS reduces motor cortex excitability and reactivity as shown by decreased MEP amplitude, increase inhibition after SICI and LICI, and a tendency towards longer cSP and less ICF. However, this reduction was only observed when using $mono_{PA}$ TMS, indicating that tSMS inhibits specific cortical interneuron networks.

2. Electroencephalographic recordings quantified an increase in spontaneous oscillatory activity in the range of beta frequencies in fronto-central regions after real tSMS. This increase was

observed in all subjects of the sample regardless of the TMS technical aspects for EMG evaluation.

3. Moreover, the increase in beta frequencies was negatively associated with the MEP inhibition captured by $mono_{PA}$. MEP amplitudes of other waveforms were not related to the beta increase, reinforcing the hypothesis that inhibitory processes are a product of the influence of tSMS on specific neural populations.

Bibliography

Aguila, J., Cudeiro, J., & Rivadulla, C. (2016). Effects of Static Magnetic Fields on the Visual Cortex: reversible Visual Deficits and Reduction of Neuronal Activity. *Cerebral Cortex (New York, N.Y. : 1991)*, *26*(2), 628–638. https://doi.org/10.1093/cercor/bhu228

Ahdab, R., Ayache, S. S., Brugieres, P., Farhat, W. H., & Lefaucheur, J.-P. (2016). The Hand Motor Hotspot is not Always Located in the Hand Knob: A Neuronavigated Transcranial Magnetic Stimulation Study. *Brain Topography*, *29*(4), 590–597. https://doi.org/10.1007/s10548-016-0486-2

Amassian, V. E., Cracco, R. Q., & Maccabee, P. J. (1989). Focal stimulation of human cerebral cortex with the magnetic coil: a comparison with electrical stimulation. *Electroencephalography and Clinical Neurophysiology*, *74*(6), 401–416.

Amassian, V. E., Quirk, G. J., & Stewart, M. (1990). A comparison of corticospinal activation by magnetic coil and electrical stimulation of monkey motor cortex. *Electroencephalography and Clinical Neurophysiology*, *77*(5), 390–401.

Amassian, V. E., Stewart, M., Quirk, G. J., & Rosenthal, J. L. (1987). Physiological basis of motor effects of a transient stimulus to cerebral cortex. *Neurosurgery*, *20*(1), 74–93.

Arias, P., Adan-Arcay, L., Puerta-Catoira, B., Madrid, A., & Cudeiro, J. (2017). Transcranial static magnetic field stimulation of M1 reduces corticospinal excitability without distorting sensorimotor integration in humans. *Brain Stimulation*, *10*(2), 340–342. https://doi.org/10.1016/j.brs.2017.01.002

Arrubla, J., Neuner, I., Hahn, D., Boers, F., & Shah, N. J. (2013). Recording visual evoked potentials and auditory evoked P300 at 9.4T static magnetic field. *PloS One*, *8*(5), e62915. https://doi.org/10.1371/journal.pone.0062915

Babiloni, C., Lizio, R., Marzano, N., Capotosto, P., Soricelli, A., Triggiani, A. I., … Del Percio, C. (2016). Brain neural synchronization and functional coupling in Alzheimer's disease as revealed by resting state EEG rhythms. *International Journal of Psychophysiology : Official Journal of the International Organization of Psychophysiology*, *103*, 88–102. https://doi.org/10.1016/j.ijpsycho.2015.02.008

Balcavage, W. X., Alvager, T., Swez, J., Goff, C. W., Fox, M. T., Abdullyava, S., & King, M. W. (1996). A mechanism for action of extremely low frequency electromagnetic fields on biological systems. *Biochemical and Biophysical Research Communications*, *222*(2), 374–378. https://doi.org/10.1006/bbrc.1996.0751

Balla, C., Maertens de Noordhout, A., & Pepin, J. L. (2014). Motor cortex excitability changes in mild Alzheimer's disease are reversed by donepezil. *Dementia and Geriatric Cognitive Disorders*, *38*(3–4), 264–270. https://doi.org/10.1159/000360617

Balslev, D., Braet, W., McAllister, C., & Miall, R. C. (2007). Inter-individual variability in optimal current direction for transcranial magnetic stimulation of the motor cortex. *Journal of Neuroscience Methods*, *162*(1–2), 309–313. https://doi.org/10.1016/j.jneumeth.2007.01.021

Barker, A. (2017). Transcranial Magnetic Stimulation-past, present and future. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, *10*(2), 540. https://doi.org/10.1016/j.brs.2017.01.573

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive Magnetic Stimulation of human motor cortex. *Originally Published as Volume 1, Issue 8437*, *325*(8437), 1106–1107. https://doi.org/10.1016/S0140-6736(85)92413-4

Bastani, A., & Jaberzadeh, S. (2012). A higher number of TMS-elicited MEP from a combined hotspot improves intra- and inter-session reliability of the upper limb muscles in healthy individuals. *PloS One*, *7*(10), e47582. https://doi.org/10.1371/journal.pone.0047582

Baugh, F. (2002). Correcting Effect Sizes for Score Reliability: A Reminder that Measurement and Substantive Issues are Linked Inextricably. *Correcting Effect Sizes for Score Reliability: A Reminder That Measurement and Substantive Issues Are Linked Inextricably*, *62*(2), 254–263. https://doi.org/10.1177/0013164402062002004

Beaulieu, L.-D., Flamand, V. H., Masse-Alarie, H., & Schneider, C. (2017). Reliability and minimal detectable change of transcranial magnetic stimulation outcomes in healthy adults: A systematic review. *Brain Stimulation*, *10*(2), 196–213. https://doi.org/10.1016/j.brs.2016.12.008

Beischer, D. E., & Knepton Jr, J. C. (1966). *The electroencephalogram of the squirrel monkey (Saimiri sciureus) in a very high magnetic field*. Naval Aerospace Medical Institute, Naval Aviation Medical Center.

Berger, B., Minarik, T., Liuzzi, G., Hummel, F. C., & Sauseng, P. (2014). EEG oscillatory phase-dependent markers of corticospinal excitability in the resting brain. *BioMed Research International*, *2014*, 936096. https://doi.org/10.1155/2014/936096

Biabani, M., Farrell, M., Zoghi, M., Egan, G., & Jaberzadeh, S. (2018). The minimal number of TMS trials required for the reliable assessment of corticospinal excitability, short interval intracortical inhibition, and intracortical facilitation. *Neuroscience Letters*, *674*, 94–100. https://doi.org/10.1016/j.neulet.2018.03.026

Bliss, T. V. P., & Cooke, S. F. (2011). Long-term potentiation and long-term depression: a clinical perspective. *Clinics*, *66*(Suppl 1), 3–17. https://doi.org/10.1590/S1807-59322011001300002

Bliss, T. V. P., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *The Journal of Physiology*, *232*(2), 331–356.

Bonnefond, M., Kastner, S., & Jensen, O. (2017). Communication between Brain Areas Based on Nested Oscillations. *ENeuro*, *4*(2). https://doi.org/10.1523/ENEURO.0153-16.2017

Brasil-Neto, J. P., Cohen, L. G., Panizza, M., Nilsson, J., Roth, B. J., & Hallett, M. (1992). Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. *Journal of Clinical Neurophysiology : Official Publication of the American Electroencephalographic Society*, *9*(1), 132–136.

Brasil-Neto, J. P., Cohen, L. G., Pascual-Leone, A., Jabir, F. K., Wall, R. T., & Hallett, M. (1992). Rapid reversible modulation of human motor outputs after transient deafferentation of the forearm: a study with transcranial magnetic stimulation. *Neurology*, *42*(7), 1302–1306.

Brem, A.-K., Atkinson, N. J., Seligson, E. E., & Pascual-Leone, A. (2013). Differential pharmacological effects on brain reactivity and plasticity in Alzheimer's disease. *Frontiers in Psychiatry*, *4*, 124. https://doi.org/10.3389/fpsyt.2013.00124

Brown, P. (2007). Abnormal oscillatory synchronisation in the motor system leads to impaired movement. *Current Opinion in Neurobiology*, *17*(6), 656–664. https://doi.org/10.1016/j.conb.2007.12.001

Bullmore, E. T., Suckling, J., Overmeyer, S., Rabe-Hesketh, S., Taylor, E., & Brammer, M. J. (1999). Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain. *IEEE Transactions on Medical Imaging*, *18*(1), 32–42.

Burke, D., Hicks, R., Gandevia, S. C., Stephen, J., Woodforth, I., & Crawford, M. (1993). Direct comparison of corticospinal volleys in human subjects to transcranial magnetic and electrical stimulation. *The Journal of Physiology*, *470*, 383–393.

Buzsaki, G. (2005). Theta rhythm of navigation: link between path integration and landmark navigation, episodic and semantic memory. *Hippocampus*, *15*(7), 827–840. https://doi.org/10.1002/hipo.20113

Cacchio, A., Paoloni, M., Cimini, N., Mangone, M., Liris, G., Aloisi, P., … Marrelli, A. (2011). Reliability of TMS-related measures of tibialis anterior muscle in patients with chronic stroke and healthy subjects. *Journal of the Neurological Sciences*, *303*(1–2), 90–94. https://doi.org/10.1016/j.jns.2011.01.004

Carrasco-Lopez, C., Soto-Leon, V., Cespedes, V., Profice, P., Strange, B. A., Foffani, G., & Oliviero, A. (2017). Static Magnetic Field Stimulation over Parietal Cortex Enhances Somatosensory Detection in Humans. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *37*(14), 3840–3847. https://doi.org/10.1523/JNEUROSCI.2123-16.2017

Carroll, T. J., Riek, S., & Carson, R. G. (2001). Reliability of the input-output properties of the cortico-spinal pathway obtained from transcranial magnetic and electrical stimulation. *Journal of Neuroscience Methods*, *112*(2), 193–202.

Cash, R. F. H., Noda, Y., Zomorrodi, R., Radhu, N., Farzan, F., Rajji, T. K., … Blumberger, D. M. (2017). Characterization of Glutamatergic and GABAA-Mediated Neurotransmission in Motor and Dorsolateral Prefrontal Cortex Using Paired-Pulse TMS-EEG. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *42*(2), 502–511. https://doi.org/10.1038/npp.2016.133

Cengiz, B., Murase, N., & Rothwell, J. C. (2013). Opposite effects of weak transcranial direct current stimulation on different phases of short interval intracortical inhibition (SICI). *Experimental Brain Research*, *225*(3), 321–331. https://doi.org/10.1007/s00221-012-3369-0

Chang, W. H., Bang, O. Y., Shin, Y.-I., Lee, A., Pascual-Leone, A., & Kim, Y.-H. (2014). BDNF polymorphism and differential rTMS effects on motor recovery of stroke patients. *Brain Stimulation*, *7*(4), 553–558. https://doi.org/10.1016/j.brs.2014.03.008

Chang, W. H., Fried, P. J., Saxena, S., Jannati, A., Gomes-Osman, J., Kim, Y.-H., & Pascual-Leone, A. (2016). Optimal number of pulses as outcome measures of neuronavigated transcranial magnetic stimulation. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *127*(8), 2892–2897. https://doi.org/10.1016/j.clinph.2016.04.001

Cheeran, B., Talelli, P., Mori, F., Koch, G., Suppa, A., Edwards, M., … Rothwell, J. C. (2008). A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. *The Journal of Physiology*, *586*(23), 5717–5725. https://doi.org/10.1113/jphysiol.2008.159905

Chen, R., Spencer, D. C., Weston, J., & Nolan, S. J. (2016). Transcranial magnetic stimulation for the treatment of epilepsy. *The Cochrane Database of Systematic Reviews*, (8), CD011025. https://doi.org/10.1002/14651858.CD011025.pub2

Cherubini, E. (2010). Phasic GABAA-mediated inhibition. *Epilepsia*, *51*, 13–13.

Christie, A., Fling, B., Crews, R. T., Mulwitz, L. A., & Kamen, G. (2007). Reliability of motor-evoked potentials in the ADM muscle of older adults. *Journal of Neuroscience Methods*, *164*(2), 320–324. https://doi.org/10.1016/j.jneumeth.2007.05.011

Chu, J., Gunraj, C., & Chen, R. (2008). Possible differences between the time courses of presynaptic and postsynaptic GABAB mediated inhibition in the human motor cortex. *Experimental Brain Research*, *184*(4), 571–577. https://doi.org/10.1007/s00221-007-1125-7

Cirillo, J., Hughes, J., Ridding, M., Thomas, P. Q., & Semmler, J. G. (2012). Differential modulation of motor cortex excitability in BDNF Met allele carriers following experimentally induced and use-dependent plasticity. *The European Journal of Neuroscience*, *36*(5), 2640–2649. https://doi.org/10.1111/j.1460-9568.2012.08177.x

Cirillo, J., & Perez, M. A. (2015). Subcortical contribution to late TMS-induced I-waves in intact humans. *Frontiers in Integrative Neuroscience*, *9*, 38. https://doi.org/10.3389/fnint.2015.00038

Cohen, J. (1992). A power primer. *Psychological Bulletin*, *112*(1), 155–159.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. Hillsdale, N.J.: L. Erlbaum Associates.

Coots, A., Shi, R., & Rosen, A. D. (2004). Effect of a 0.5-T static magnetic field on conduction in guinea pig spinal cord. *Journal of the Neurological Sciences*, *222*(1–2), 55–57. https://doi.org/10.1016/j.jns.2004.04.010

Danner, N., Julkunen, P., Kononen, M., Saisanen, L., Nurkkala, J., & Karhu, J. (2008). Navigated transcranial magnetic stimulation and computed electric field strength reduce stimulator-dependent differences in the motor threshold. *Journal of Neuroscience Methods*, *174*(1), 116–122. https://doi.org/10.1016/j.jneumeth.2008.06.032

Day, B. L., Dressler, D., Noordhout, A. M. de, Marsden, C. D., Nakashima, K., Rothwell, J. C., & Thompson, P. D. (1989). Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. *Electric and Magnetic Stimulation of Human Motor*

Cortex: Surface EMG and Single Motor Unit Responses., *412*(1), 449–473. https://doi.org/10.1113/jphysiol.1989.sp017626

De Gennaro, L., Ferrara, M., Bertini, M., Pauri, F., Cristiani, R., Curcio, G., … Rossini, P. M. (2003). Reproducibility of callosal effects of transcranial magnetic stimulation (TMS) with interhemispheric paired pulses. *Neuroscience Research*, *46*(2), 219–227. https://doi.org/10.1016/S0168-0102(03)00060-9

Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods*, *134*(1), 9–21. https://doi.org/10.1016/j.jneumeth.2003.10.009

Delvendahl, I., Gattinger, N., Berger, T., Gleich, B., Siebner, H. R., & Mall, V. (2014). The role of pulse shape in motor cortex transcranial magnetic stimulation using full-sine stimuli. *PloS One*, *9*(12), e115247. https://doi.org/10.1371/journal.pone.0115247

Delvendahl, I., Lindemann, H., Jung, N. H., Pechmann, A., Siebner, H. R., & Mall, V. (2014). Influence of Waveform and Current Direction on Short-Interval Intracortical Facilitation: A Paired-Pulse TMS Study. *Brain Stimulation*, *7*(1), 49–58. https://doi.org/10.1016/j.brs.2013.08.002

Deng, Z.-D., Lisanby, S. H., & Peterchev, A. V. (2010). Transcranial magnetic stimulation in the presence of deep brain stimulation implants: Induced electrode currents. *Conference Proceedings : ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual Conference*, *2010*, 6821–6824. https://doi.org/10.1109/IEMBS.2010.5625958

Deuschl, G., & Eisen, A. (1999). Recommendations for the practice of clinical neurophysiology: guidelines of the International Federation of Clinical Neurophysiology.

Di Lazzaro, V., Oliviero, A., Mazzone, P., Insola, A., Pilato, F., Saturno, E., … Rothwell, J. (2001). Comparison of descending volleys evoked by monophasic and biphasic magnetic stimulation of the motor cortex in conscious humans. *Experimental Brain Research*, *141*(1), 121–127. https://doi.org/10.1007/s002210100863

Di Lazzaro, V., Oliviero, A., Mazzone, P., Pilato, F., Saturno, E., Insola, A., … Rothwell, J. C. (2002). Direct demonstration of long latency cortico-cortical inhibition in normal subjects and in a patient with vascular parkinsonism. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *113*(11), 1673–1679. https://doi.org/10.1016/S1388-2457(02)00264-X

Di Lazzaro, V., Oliviero, A., Meglio, M., Cioni, B., Tamburrini, G., Tonali, P., & Rothwell, J. C. (2000). Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *111*(5), 794–799. https://doi.org/10.1016/S1388-2457(99)00314-4

Di Lazzaro, V., Oliviero, A., Pilato, F., Mazzone, P., Insola, A., Ranieri, F., & Tonali, P. A. (2003). Corticospinal volleys evoked by transcranial stimulation of the brain in conscious humans. *Neurological Research*, *25*(2), 143–150. https://doi.org/10.1179/016164103101201292

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Mazzone, P., … Rothwell, J. C. (2004). The physiological basis of transcranial motor cortex stimulation in conscious humans. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *115*(2), 255–266. https://doi.org/10.1016/j.clinph.2003.10.009

Di Lazzaro, V., Oliviero, A., Profice, P., Saturno, E., Pilato, F., Insola, A., … Rothwell, J. C. (1998). Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. *Electroencephalography and Clinical Neurophysiology*, *109*(5), 397–401.

Di Lazzaro, V., Oliviero, A., Saturno, E., Pilato, F., Insola, A., Mazzone, P., … Rothwell, J. (2001). The effect on corticospinal volleys of reversing the direction of current induced in the motor cortex by transcranial magnetic stimulation. *Experimental Brain Research*, *138*(2), 268–273. https://doi.org/10.1007/s002210100722

Di Lazzaro, V., Pellegrino, G., Di Pino, G., Corbetto, M., Ranieri, F., Brunelli, N., … Capone, F. (2015). Val66Met BDNF gene polymorphism influences human motor cortex plasticity in acute stroke. *Brain Stimulation*, *8*(1), 92–96. https://doi.org/10.1016/j.brs.2014.08.006

Di Lazzaro, V., Pilato, F., Oliviero, A., Dileone, M., Saturno, E., Mazzone, P., … Rothwell, J. C. (2006). Origin of facilitation of motor-evoked potentials after paired magnetic stimulation: direct recording of epidural activity in conscious humans. *Journal of Neurophysiology*, *96*(4), 1765–1771. https://doi.org/10.1152/jn.00360.2006

Di Lazzaro, V., Profice, P., Ranieri, F., Capone, F., Dileone, M., Oliviero, A., & Pilato, F. (2011). I-wave origin and modulation. *Brain Stimulation*, *5*(4). https://doi.org/10.1016/j.brs.2011.07.008

Di Lazzaro, V., Restuccia, D., Oliviero, A., Profice, P., Ferrara, L., Insola, A., … Rothwell, J. C. (1998). Magnetic transcranial stimulation at intensities below active motor threshold activates intracortical inhibitory circuits. *Experimental Brain Research*, *119*(2), 265–268.

Di Lazzaro, V., & Rothwell, J. C. (2014). Corticospinal activity evoked and modulated by non-invasive stimulation of the intact human motor cortex. *The Journal of Physiology*, *592*(Pt 19), 4115–4128. https://doi.org/10.1113/jphysiol.2014.274316

Di Lazzaro, V., Rothwell, J., & Capogna, M. (2017). Noninvasive Stimulation of the Human Brain: Activation of Multiple Cortical Circuits. *The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, 1073858417717660. https://doi.org/10.1177/1073858417717660

Di Lazzaro, V., & Ziemann, U. (2013). The contribution of transcranial magnetic stimulation in the functional evaluation of microcircuits in human motor cortex. *Front Neural Circuits*, *7*, 18. https://doi.org/10.3389/fncir.2013.00018

Di Lazzaro, V., Ziemann, U., & Lemon, R. N. (2008). State of the art: Physiology of transcranial motor cortex stimulation. *Brain Stimulation*, *1*(4), 345–362. https://doi.org/10.1016/j.brs.2008.07.004

Di Lorenzo, F., Ponzo, V., Bonni, S., Motta, C., Negrao Serra, P. C., Bozzali, M., … Koch, G. (2016). Long-term potentiation-like cortical plasticity is disrupted in Alzheimer's disease patients independently from age of onset. *Annals of Neurology*, *80*(2), 202–210. https://doi.org/10.1002/ana.24695

Diamond, D. M., Dunwiddie, T. V., & Rose, G. M. (1988). Characteristics of hippocampal primed burst potentiation in vitro and in the awake rat. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *8*(11), 4079–4088.

Dileone, M., Carrasco-Lopez, M. C., Segundo-Rodriguez, J. C., Mordillo-Mateos, L., Lopez-Ariztegui, N., Alonso-Frech, F., … Foffani, G. (2017). Dopamine-dependent changes of cortical excitability induced by transcranial static magnetic field stimulation in Parkinson's disease. *Scientific Reports*, *7*(1), 4329. https://doi.org/10.1038/s41598-017-04254-y

Dobson, J., & St Pierre, T. (1996). Application of the ferromagnetic transduction model to D.C. and pulsed magnetic fields: effects on epileptogenic tissue and implications for cellular phone safety. *Biochemical and Biophysical Research Communications*, *227*(3), 718–723.

D'Ostilio, K., Goetz, S. M., Hannah, R., Ciocca, M., Chieffo, R., Chen, J.-C. A., … Rothwell, J. C. (2016). Effect of coil orientation on strength–duration time constant and I-wave activation with controllable pulse parameter transcranial magnetic stimulation. *Clinical Neurophysiology*, *127*(1), 675–683. https://doi.org/10.1016/j.clinph.2015.05.017

Engel, A. K., & Fries, P. (2010). Beta-band oscillations—signalling the status quo? *Beta-Band Oscillations—Signalling the Status Quo?* *20*(2), 156–165. https://doi.org/10.1016/j.conb.2010.02.015

Espenhahn, S., de Berker, A. O., van Wijk, B. C., Rossiter, H. E., & Ward, N. S. (2017). Movement-related beta oscillations show high intra-individual reliability. *Neuroimage*, *147*, 175–185. https://doi.org/10.1016/j.neuroimage.2016.12.025

Farzan, F., Barr, M. S., Levinson, A. J., Chen, R., Wong, W., Fitzgerald, P. B., & Daskalakis, Z. J. (2010). Reliability of long-interval cortical inhibition in healthy human subjects: a TMS-EEG study. *Journal of Neurophysiology*, *104*(3), 1339–1346. https://doi.org/10.1152/jn.00279.2010

Fleming, M. K., Sorinola, I. O., Newham, D. J., Roberts-Lewis, S. F., & Bergmann, J. H. M. (2012). The Effect of Coil Type and Navigation on the Reliability of Transcranial Magnetic Stimulation. *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, *20*(5), 617625. https://doi.org/10.1109/TNSRE.2012.2202692

Foucher, J., Lorgouillou, K., Turek, J., Pham, B.-T., Elowe, J., Bayle, B., … Armspach, J. (2012). *Robotic assistance in coil positioning improves reliability and comfort*.

Freitas, C., Perez, J., Knobel, M., Tormos, J. M., Oberman, L., Eldaief, M., … Pascual-Leone, A. (2011). Changes in Cortical Plasticity Across the Lifespan. *Changes in Cortical Plasticity Across the Lifespan*, *3*, 5. https://doi.org/10.3389/fnagi.2011.00005

Fried, P. J., Schilberg, L., Brem, A.-K., Saxena, S., Wong, B., Cypess, A. M., … Pascual-Leone, A. (2017). Humans with Type-2 Diabetes Show Abnormal Long-Term Potentiation-Like Cortical Plasticity Associated with Verbal Learning Deficits. *Journal of Alzheimer's Disease : JAD*, *55*(1), 89–100. https://doi.org/10.3233/JAD-160505

Friedman, H. (1968). Magnitude of experimental effect and a table for its rapid estimation. *Psychological Bulletin*, *70*(4), 245.

Fries, P. (2005). A mechanism for cognitive dynamics: neuronal communication through neuronal coherence. *Trends in Cognitive Sciences*, *9*(10), 474–480. https://doi.org/10.1016/j.tics.2005.08.011

Fries, P. (2015). Rhythms for Cognition: Communication through Coherence. *Rhythms for Cognition: Communication through Coherence*, *88*(1), 220–235. https://doi.org/10.1016/j.neuron.2015.09.034

Fuhr, P., Cohen, L. G., Roth, B. J., & Hallett, M. (1991). Latency of motor evoked potentials to focal transcranial stimulation varies as a function of scalp positions stimulated. *Electroencephalography and Clinical Neurophysiology*, *81*(2), 81–89.

Gamboa, O., Antal, A., Moliadze, V., & Paulus, W. (2010). Simply longer is not better: reversal of theta burst after-effect with prolonged stimulation. *Experimental Brain Research*, *204*(2), 181–187. https://doi.org/10.1007/s00221-010-2293-4

Gilbertson, T., Lalo, E., Doyle, L., Di Lazzaro, V., Cioni, B., & Brown, P. (2005). Existing motor state is favored at the expense of new movement during 13-35 Hz oscillatory synchrony in the human corticospinal system. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *25*(34), 7771–7779. https://doi.org/10.1523/JNEUROSCI.1762-05.2005

Ginhoux, R., Renaud, P., Zorn, L., Goffin, L., Bayle, B., Foucher, J., … de Mathelin, M. (2013). A custom robot for Transcranial Magnetic Stimulation: first assessment on healthy subjects. *Conference Proceedings : ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual Conference*, *2013*, 5352–5355. https://doi.org/10.1109/EMBC.2013.6610758

Gispen, W. H., & Biessels, G. J. (2000). Cognition and synaptic plasticity in diabetes mellitus. *Trends in Neurosciences*, *23*(11), 542–549. https://doi.org/10.1016/S0166-2236(00)01656-8

Goldsworthy, M. R., Hordacre, B., & Ridding, M. C. (2016). Minimum number of trials required for within- and between-session reliability of TMS measures of corticospinal excitability. *Minimum Number of Trials Required for Within- and between-Session Reliability of TMS Measures of Corticospinal Excitability*, *320*, 205–209. https://doi.org/10.1016/j.neuroscience.2016.02.012

Goldsworthy, M. R., Muller-Dahlhaus, F., Ridding, M. C., & Ziemann, U. (2014). Inter-subject variability of LTD-like plasticity in human motor cortex: a matter of preceding motor activation. *Brain Stimulation*, *7*(6), 864–870. https://doi.org/10.1016/j.brs.2014.08.004

Gonzalez-Rosa, J. J., Soto-León, V., Real, P., Carrasco-López, M. C., Foffani, G., Strange, B. A., & Oliviero, A. (2015). Static Magnetic Field Stimulation over the Visual Cortex Increases Alpha Oscillations and Slows Visual Search in Humans. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *35*(24), 9182–9193. https://doi.org/10.1523/JNEUROSCI.4232-14.2015

Gottmann, K., Mittmann, T., & Lessmann, V. (2009). BDNF signaling in the formation, maturation and plasticity of glutamatergic and GABAergic synapses. *Experimental Brain Research*, *199*(3–4), 203–234. https://doi.org/10.1007/s00221-009-1994-z

Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L. G., Mall, V., … Siebner, H. R. (2012). A practical guide to diagnostic transcranial magnetic stimulation: Report of an IFCN committee. *Clinical Neurophysiology*, *123*(5), 858882. https://doi.org/10.1016/j.clinph.2012.01.010

Groppe, D. M., Urbach, T. P., & Kutas, M. (2011). Mass univariate analysis of event-related brain potentials/fields II: Simulation studies. *Psychophysiology*, *48*(12), 1726–1737. https://doi.org/10.1111/j.1469-8986.2011.01272.x

Hallett, M. (2007). Transcranial magnetic stimulation: a primer. *Neuron*, *55*(2), 187–199. https://doi.org/10.1016/j.neuron.2007.06.026

Hallett, M., & Chokroverty, S. (2005). Magnetic stimulation in clinical neurophysiology. *Magnetic Stimulation in Clinical Neurophysiology*. Retrieved from http://books.google.com/books?hl=en&lr=&id=5v9gY96bVXcC&oi=fnd&pg=PR12&dq=0-7506-7373-7&ots=fgUeexwo1F&sig=HcOs8bJEBJlhUXA2kWWo9fcSmf4

Hameed, M. Q., Dhamne, S. C., Gersner, R., Kaye, H. L., Oberman, L. M., Pascual-Leone, A., & Rotenberg, A. (2017). Transcranial Magnetic and Direct Current Stimulation in Children. *Current Neurology and Neuroscience Reports*, *17*(2), 11. https://doi.org/10.1007/s11910-017-0719-0

Hannah, R., & Rothwell, J. C. (2017). Pulse Duration as Well as Current Direction Determines the Specificity of Transcranial Magnetic Stimulation of Motor Cortex during Contraction. *Brain Stimulation*, *10*(1), 106–115. https://doi.org/10.1016/j.brs.2016.09.008

Hermsen, A. M., Haag, A., Duddek, C., Balkenhol, K., Bugiel, H., Bauer, S., … Rosenow, F. (2016). Test–retest reliability of single and paired pulse transcranial magnetic stimulation parameters in healthy subjects. *J Neurol Sci*, *362*, 209–216. https://doi.org/10.1016/j.jns.2016.01.039

Herwig, U., Satrapi, P., & Schonfeldt-Lecuona, C. (2003). Using the international 10-20 EEG system for positioning of transcranial magnetic stimulation. *Brain Topography*, *16*(2), 95–99.

Herwig, U., Schonfeldt-Lecuona, C., Wunderlich, A. P., von Tiesenhausen, C., Thielscher, A., Walter, H., & Spitzer, M. (2001). The navigation of transcranial magnetic stimulation. *Psychiatry Research*, *108*(2), 123–131. https://doi.org/10.1016/S0925-4927(01)00121-4

Hinder, M. R., Goss, E. L., Fujiyama, H., Canty, A. J., Garry, M. I., Rodger, J., & Summers, J. J. (2014). Inter- and Intra-individual variability following intermittent theta burst stimulation:

implications for rehabilitation and recovery. *Brain Stimulation*, *7*(3), 365–371. https://doi.org/10.1016/j.brs.2014.01.004

Holland, R. A., Thorup, K., Vonhof, M. J., Cochran, W. W., & Wikelski, M. (2006). Navigation: bat orientation using Earth's magnetic field. *Nature*, *444*(7120), 702. https://doi.org/10.1038/444702a

Hölscher, C. (2011). Diabetes as a risk factor for Alzheimer's disease: insulin signalling impairment in the brain as an alternative model of Alzheimer's disease. *Biochemical Society Transactions*, *39*(4), 891–897. https://doi.org/10.1042/BST0390891

Hoonhorst, M. H. W. J., Kollen, B. J., Berg, P. S. P. van den, Emmelot, C. H., & Kwakkel, G. (2014). How reproducible are transcranial magnetic stimulation-induced MEPs in subacute stroke? *How Reproducible Are Transcranial Magnetic Stimulation-Induced MEPs in Subacute Stroke?* *31*(6), 556–562. https://doi.org/10.1097/wnp.0000000000000114

Hordacre, B., Ridding, M., & Goldsworthy, M. (2015). Response variability to non-invasive brain stimulation protocols. *Clin Neurophysiol*, *126*(12), 2249–2250. https://doi.org/10.1016/j.clinph.2015.04.052

Huang, Y. Z., Trender-Gerhard, I., Edwards, M. J., & Mir, P. (2006). Motor system inhibition in dopa-responsive dystonia and its modulation by treatment. https://doi.org/10.1212/01.wnl.0000214304.03105.f4

Huang, Y.-Z., Edwards, M. J., Rounis, E., Bhatia, K. P., & Rothwell, J. C. (2004). Theta Burst Stimulation of the Human Motor Cortex. *Neuron*, *45*(2). https://doi.org/10.1016/j.neuron.2004.12.033

Huang, Y.-Z., Rothwell, J. C., Edwards, M. J., & Chen, R.-S. (2008). Effect of physiological activity on an NMDA-dependent form of cortical plasticity in human. *Cerebral Cortex (New York, N.Y. : 1991)*, *18*(3), 563–570. https://doi.org/10.1093/cercor/bhm087

Hyvarinen, A., & Oja, E. (2000). Independent component analysis: algorithms and applications. *Neural Networks : The Official Journal of the International Neural Network Society*, *13*(4–5), 411–430.

Iezzi, E., Conte, A., Suppa, A., Agostino, R., Dinapoli, L., Scontrini, A., & Berardelli, A. (2008). Phasic voluntary movements reverse the aftereffects of subsequent theta-burst stimulation in humans. *Journal of Neurophysiology*, *100*(4), 2070–2076. https://doi.org/10.1152/jn.90521.2008

Inghilleri, M., Berardelli, A., Cruccu, G., & Manfredi, M. (1993). Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. *J Physiology*, *466*(1), 521–534. https://doi.org/10.1113/jphysiol.1993.sp019732

Iscan, Z., Nazarova, M., Fedele, T., Blagovechtchenski, E., & Nikulin, V. V. (2016). Pre-stimulus Alpha Oscillations and Inter-subject Variability of Motor Evoked Potentials in Single- and Paired-Pulse TMS Paradigms. *Frontiers in Human Neuroscience*, *10*, 504. https://doi.org/10.3389/fnhum.2016.00504

Jenkinson, N., & Brown, P. (2011). New insights into the relationship between dopamine, beta oscillations and motor function. *Trends in Neurosciences*, *34*(12), 611–618. https://doi.org/10.1016/j.tins.2011.09.003

Julkunen, P., Saisanen, L., Danner, N., Niskanen, E., Hukkanen, T., Mervaala, E., & Kononen, M. (2009). Comparison of navigated and non-navigated transcranial magnetic stimulation for motor cortex mapping, motor threshold and motor evoked potentials. *NeuroImage*, *44*(3), 790–795. https://doi.org/10.1016/j.neuroimage.2008.09.040

Kamen, G. (2004). Reliability of motor-evoked potentials during resting and active contraction conditions. *Medicine and Science in Sports and Exercise*, *36*(9), 1574–1579. https://doi.org/10.1249/01.MSS.0000139804.02576.6A

Kammer, T., Beck, S., Thielscher, A., Laubis-Herrmann, U., & Topka, H. (2001). Motor thresholds in humans: a transcranial magnetic stimulation study comparing different pulse waveforms, current directions and stimulator types. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *112*(2), 250–258. https://doi.org/10.1016/S1388-2457(00)00513-7

Kanai, R., Chaieb, L., Antal, A., Walsh, V., & Paulus, W. (2008). Frequency-dependent electrical stimulation of the visual cortex. *Current Biology : CB*, *18*(23), 1839–1843. https://doi.org/10.1016/j.cub.2008.10.027

Kantelhardt, S. R., Fadini, T., Finke, M., Kallenberg, K., Siemerkus, J., Bockermann, V., … Giese, A. (2010). Robot-assisted image-guided transcranial magnetic stimulation for somatotopic mapping of the motor cortex: a clinical pilot study. *Acta Neurochirurgica*, *152*(2), 333–343. https://doi.org/10.1007/s00701-009-0565-1

Kenny, D. A., Mannetti, L., Pierro, A., Livi, S., & Kashy, D. A. (2002). The statistical analysis of data from small groups. *Journal of Personality and Social Psychology*, *83*(1), 126–137. https://doi.org/10.1037/0022-3514.83.1.126

Kim, S. J., & Kim, K. A. (2017). Safety issues and updates under MR environments. *European Journal of Radiology*, *89*, 7–13. https://doi.org/10.1016/j.ejrad.2017.01.010

Kimiskidis, V. K., Papagiannopoulos, S., Sotirakoglou, K., Kazis, D. A., Dimopoulos, G., Kazis, A., & Mills, K. R. (2004). The repeatability of corticomotor threshold measurements. *Neurophysiologie Clinique = Clinical Neurophysiology*, *34*(6), 259–266. https://doi.org/10.1016/j.neucli.2004.10.002

Kirimoto, H., Asao, A., Tamaki, H., & Onishi, H. (2016). Non-invasive modulation of somatosensory evoked potentials by the application of static magnetic fields over the primary and supplementary motor cortices. *Non-Invasive Modulation of Somatosensory Evoked Potentials by the Application of Static Magnetic Fields over the Primary and Supplementary Motor Cortices*, *6*(1), 34509. https://doi.org/10.1038/srep34509

Kirimoto, H., Tamaki, H., Matsumoto, T., Sugawara, K., Suzuki, M., Oyama, M., & Onishi, H. (2014). Effect of Transcranial Static Magnetic Field Stimulation Over the Sensorimotor Cortex on Somatosensory Evoked Potentials in Humans. *Brain Stimulation*, *7*(6), 836–840. https://doi.org/10.1016/j.brs.2014.09.016

Koch, G. (2010). Repetitive transcranial magnetic stimulation: a tool for human cerebellar plasticity. *Functional Neurology*, *25*(3), 159–163.

Koch, G., Di Lorenzo, F., Bonni, S., Ponzo, V., Caltagirone, C., & Martorana, A. (2012). Impaired LTP- but not LTD-like cortical plasticity in Alzheimer's disease patients. *Journal of Alzheimer's Disease : JAD*, *31*(3), 593–599. https://doi.org/10.3233/JAD-2012-120532

Koch, G., Di Lorenzo, F., Del Olmo, M. F., Bonni, S., Ponzo, V., Caltagirone, C., … Martorana, A. (2016). Reversal of LTP-Like Cortical Plasticity in Alzheimer's Disease Patients with Tau-Related Faster Clinical Progression. *Journal of Alzheimer's Disease : JAD*, *50*(2), 605–616. https://doi.org/10.3233/JAD-150813

Kozel, F. A., Nahas, Z., deBrux, C., Molloy, M., Lorberbaum, J. P., Bohning, D., … George, M. S. (2000). How coil-cortex distance relates to age, motor threshold, and antidepressant response to repetitive transcranial magnetic stimulation. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *12*(3), 376–384. https://doi.org/10.1176/jnp.12.3.376

Kujirai, T., Caramia, M. D., Rothwell, J. C., Day, B. L., Thompson, P. D., Ferbert, A., … Marsden, C. D. (1993). Corticocortical inhibition in human motor cortex. *The Journal of Physiology*, *471*(1), 501–519. https://doi.org/10.1113/jphysiol.1993.sp019912

Lang, N., Harms, J., Weyh, T., Lemon, R. N., Paulus, W., Rothwell, J. C., & Siebner, H. R. (2006). Stimulus intensity and coil characteristics influence the efficacy of rTMS to suppress cortical excitability. *Stimulus Intensity and Coil Characteristics Influence the Efficacy of RTMS to Suppress Cortical Excitability*, *117*(10), 2292–2301. https://doi.org/10.1016/j.clinph.2006.05.030

Lee, M., Kim, S. E., Kim, W. S., Lee, J., Yoo, H. K., Park, K.-D., … Lee, H. W. (2013). Interaction of motor training and intermittent theta burst stimulation in modulating motor cortical plasticity: influence of BDNF Val66Met polymorphism. *PloS One*, *8*(2), e57690. https://doi.org/10.1371/journal.pone.0057690

Lefaucheur, J.-P., André-Obadia, N., Antal, A., Ayache, S. S., Baeken, C., Benninger, D. H., … Garcia-Larrea, L. (2014). Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). *Clinical Neurophysiology*, *125*(11), 2150–2206. https://doi.org/10.1016/j.clinph.2014.05.021

Li Voti, P., Conte, A., Suppa, A., Iezzi, E., Bologna, M., Aniello, M. S., … Berardelli, A. (2011). Correlation between cortical plasticity, motor learning and BDNF genotype in healthy subjects. *Experimental Brain Research*, *212*(1), 91–99. https://doi.org/10.1007/s00221-011-2700-5

Liepert, J., Bär, K., Meske, U., & Weiller, C. (2001). Motor cortex disinhibition in Alzheimer's disease. *Clinical Neurophysiology*, *112*(8), 1436–1441. https://doi.org/10.1016/S1388-2457(01)00554-5

Liepert, J., Restemeyer, C., Kucinski, T., Zittel, S., & Weiller, C. (2005). Motor strokes: the lesion location determines motor excitability changes. *Stroke*, *36*(12), 2648–2653. https://doi.org/10.1161/01.STR.0000189629.10603.02

Little, S., & Brown, P. (2014). The functional role of beta oscillations in Parkinson's disease. *Parkinsonism & Related Disorders*, *20 Suppl 1*, S44-48. https://doi.org/10.1016/S1353-8020(13)70013-0

Liu, H., & Au-Yeung, S. S. Y. (2014). Reliability of transcranial magnetic stimulation induced corticomotor excitability measurements for a hand muscle in healthy and chronic stroke subjects. *Journal of the Neurological Sciences*, *341*(1–2), 105–109. https://doi.org/10.1016/j.jns.2014.04.012

Livingston, S. C., Goodkin, H. P., & Ingersoll, C. D. (2010). The influence of gender, hand dominance, and upper extremity length on motor evoked potentials. *Journal of Clinical Monitoring and Computing*, *24*(6), 427–436. https://doi.org/10.1007/s10877-010-9267-8

Livingston, S. C., & Ingersoll, C. D. (2008). Intra-rater reliability of a transcranial magnetic stimulation technique to obtain motor evoked potentials. *The International Journal of Neuroscience*, *118*(2), 239–256. https://doi.org/10.1080/00207450701668020

Lohmann, K. J. (1993). Magnetic compass orientation. *Nature*, *362*(6422), 703. https://doi.org/10.1038/362703a0

Lozano-Soto, E., Soto-Leon, V., Sabbarese, S., Ruiz-Alvarez, L., Sanchez-Del-Rio, M., Aguilar, J., … Oliviero, A. (2017). Transcranial static magnetic field stimulation (tSMS) of the visual cortex decreases experimental photophobia. *Cephalalgia : An International Journal of Headache*, 333102417736899. https://doi.org/10.1177/0333102417736899

Maccabee, P. J., Amassian, V. E., Eberle, L. P., & Cracco, R. Q. (1993). Magnetic coil stimulation of straight and bent amphibian and mammalian peripheral nerve in vitro: locus of excitation. *The Journal of Physiology*, *460*, 201–219.

Maccabee, P. J., Nagarajan, S. S., Amassian, V. E., Durand, D. M., Szabo, A. Z., Ahad, A. B., … Eberle, L. P. (1998). Influence of pulse sequence, polarity and amplitude on magnetic stimulation of human and porcine peripheral nerve. *The Journal of Physiology*, *513 (Pt 2)*, 571–585.

Maeda, F., Gangitano, M., Thall, M., & Pascual-Leone, A. (2002). Inter- and intra-individual variability of paired-pulse curves with transcranial magnetic stimulation (TMS). *Clin Neurophysiol*, *113*(3), 376–382. https://doi.org/10.1016/S1388-2457(02)00008-1

Maeda, F., Keenan, J. P., Tormos, J. M., Topka, H., & Pascual-Leone, A. (2000). Modulation of corticospinal excitability by repetitive transcranial magnetic stimulation. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *111*(5), 800–805. https://doi.org/10.1016/S1388-2457(99)00323-5

Mäki, H., & Ilmoniemi, R. (2010). EEG oscillations and magnetically evoked motor potentials reflect motor system excitability in overlapping neuronal populations. *Clin Neurophysiol*, *121*(4), 492–501. https://doi.org/10.1016/j.clinph.2009.11.078

Maris, E., & Oostenveld, R. (2007). Nonparametric statistical testing of EEG- and MEG-data. *Journal of Neuroscience Methods*, *164*(1), 177–190. https://doi.org/10.1016/j.jneumeth.2007.03.024

Mastroeni, C., Bergmann, T. O., Rizzo, V., Ritter, C., Klein, C., Pohlmann, I., … Siebner, H. R. (2013). Brain-derived neurotrophic factor--a major player in stimulation-induced homeostatic metaplasticity of human motor cortex? *PloS One*, *8*(2), e57957. https://doi.org/10.1371/journal.pone.0057957

Matsugi, A., & Okada, Y. (2017). Cerebellar transcranial static magnetic field stimulation transiently reduces cerebellar brain inhibition. *Functional Neurology*, *32*(2), 77–82.

McClintock, S. M., Freitas, C., Oberman, L., Lisanby, S. H., & Pascual-Leone, A. (2011). Transcranial magnetic stimulation: a neuroscientific probe of cortical function in schizophrenia. *Biological Psychiatry*, *70*(1), 19–27. https://doi.org/10.1016/j.biopsych.2011.02.031

McConnell, K. A., Nahas, Z., Shastri, A., Lorberbaum, J. P., Kozel, F. A., Bohning, D. E., & George, M. S. (2001). The transcranial magnetic stimulation motor threshold depends on the distance from coil to underlying cortex: a replication in healthy adults comparing two methods of assessing the distance to cortex. *Biological Psychiatry*, *49*(5), 454–459.

McDonnell, M. N., Buckley, J. D., Opie, G. M., Ridding, M. C., & Semmler, J. G. (2013). A single bout of aerobic exercise promotes motor cortical neuroplasticity. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *114*(9), 1174–1182. https://doi.org/10.1152/japplphysiol.01378.2012

McDonnell, M. N., Ridding, M. C., & Miles, T. S. (2004). Do alternate methods of analysing motor evoked potentials give comparable results? *Journal of Neuroscience Methods*, *136*(1), 63–67. https://doi.org/10.1016/j.jneumeth.2003.12.020

McGraw, K. O., & Wong, S. P. (1996). Forming inferences about some intraclass correlation coefficients. *Psychological Methods*, *1*(1), 30.

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack, C. R. J., Kawas, C. H., … Phelps, C. H. (2011). The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association*, *7*(3), 263–269. https://doi.org/10.1016/j.jalz.2011.03.005

McLean, M. J., Engstrom, S., Holcomb, R. R., & Sanchez, D. (2003). A static magnetic field modulates severity of audiogenic seizures and anticonvulsant effects of phenytoin in DBA/2 mice. *Epilepsy Research*, *55*(1–2), 105–116. https://doi.org/10.1016/S0920-1211(03)00109-8

McLean, M. J., Engstrom, S., Qinkun, Z., Spankovich, C., & Polley, D. B. (2008). Effects of a static magnetic field on audiogenic seizures in black Swiss mice. *Epilepsy Research*, *80*(2–3), 119–131. https://doi.org/10.1016/j.eplepsyres.2008.03.022

McQuail, J. A., Banuelos, C., LaSarge, C. L., Nicolle, M. M., & Bizon, J. L. (2012). GABA(B) receptor GTP-binding is decreased in the prefrontal cortex but not the hippocampus of

aged rats. *Neurobiology of Aging*, *33*(6), 1124.e1-12. https://doi.org/10.1016/j.neurobiolaging.2011.11.011

Mills, K. R., Boniface, S. J., & Schubert, M. (1992). Magnetic brain stimulation with a double coil: the importance of coil orientation. *Electroencephalography and Clinical Neurophysiology*, *85*(1), 17–21.

Mirdamadi, J. L., Suzuki, L. Y., & Meehan, S. K. (2017). Attention modulates specific motor cortical circuits recruited by transcranial magnetic stimulation. *Neuroscience*, *359*, 151–158. https://doi.org/10.1016/j.neuroscience.2017.07.028

Miyakoshi, J. (2005). Effects of static magnetic fields at the cellular level. *Progress in Biophysics and Molecular Biology*, *87*(2–3), 213223. https://doi.org/10.1016/j.pbiomolbio.2004.08.008

Mott, D. (2015). *The metabotropic GABAB receptors*. https://doi.org/10.1016/B978-0-12-397032-9.00011-X

Nakamura, H., Kitagawa, H., Kawaguchi, Y., & Tsuji, H. (1996). Direct and indirect activation of human corticospinal neurons by transcranial magnetic and electrical stimulation. *Neuroscience Letters*, *210*(1), 45–48. http://dx.doi.org/10.1016/0304-3940(96)12659-8

Neuling, T., Rach, S., & Herrmann, C. S. (2013). Orchestrating neuronal networks: sustained after-effects of transcranial alternating current stimulation depend upon brain states. *Frontiers in Human Neuroscience*, *7*, 161. https://doi.org/10.3389/fnhum.2013.00161

Ngomo, S., Leonard, G., Moffet, H., & Mercier, C. (2012). Comparison of transcranial magnetic stimulation measures obtained at rest and under active conditions and their reliability. *Journal of Neuroscience Methods*, *205*(1), 65–71. https://doi.org/10.1016/j.jneumeth.2011.12.012

Ni, Z., Charab, S., Gunraj, C., Nelson, A. J., Udupa, K., Yeh, I.-J. J., & Chen, R. (2011). Transcranial magnetic stimulation in different current directions activates separate cortical circuits. *Journal of Neurophysiology*, *105*(2), 749–756. https://doi.org/10.1152/jn.00640.2010

Niehaus, L., Meyer, B., & Weyh, T. (2000). Influence of pulse configuration and direction of coil current on excitatory effects of magnetic motor cortex and nerve stimulation. *Influence of Pulse Configuration and Direction of Coil Current on Excitatory Effects of Magnetic Motor Cortex and Nerve Stimulation.*, *111*(1), 75–80. https://doi.org/10.1016/S1388-2457(99)00198-4

Niskanen, E., Julkunen, P., Saisanen, L., Vanninen, R., Karjalainen, P., & Kononen, M. (2010). Group-level variations in motor representation areas of thenar and anterior tibial muscles: Navigated Transcranial Magnetic Stimulation Study. *Human Brain Mapping*, *31*(8), 1272–1280. https://doi.org/10.1002/hbm.20942

Nojima, I., Koganemaru, S., Fukuyama, H., & Mima, T. (2015). Static magnetic field can transiently alter the human intracortical inhibitory system. *Clinical Neurophysiology*. https://doi.org/10.1016/j.clinph.2015.01.030

Nuwer, M. R., Lehmann, D., Silva, F. L. da, Matsuoka, S., Sutherling, W., & Vibert, J.-F. (1994). IFCN guidelines for topographic and frequency analysis of EEGs and EPs. Report of an IFCN committee. *Electroencephalography and Clinical Neurophysiology*, *91*(1), 1–5. https://doi.org/10.1016/0013-4694(94)90011-6

Oberman, L., Eldaief, M., Fecteau, S., Ifert-Miller, F., Tormos, J. M., & Pascual-Leone, A. (2012). Abnormal modulation of corticospinal excitability in adults with Asperger's syndrome. *The European Journal of Neuroscience*, *36*(6), 2782–2788. https://doi.org/10.1111/j.1460-9568.2012.08172.x

Oberman, L. M., Ifert-Miller, F., Najib, U., Bashir, S., Heydrich, J. G., Picker, J., … Pascual-Leone, A. (2016). Abnormal Mechanisms of Plasticity and Metaplasticity in Autism Spectrum Disorders and Fragile X Syndrome. *Journal of Child and Adolescent Psychopharmacology*, *26*(7), 617–624. https://doi.org/10.1089/cap.2015.0166

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, *9*(1), 97–113.

Oliviero, A., Carrasco-López, M. C., Campolo, M., Perez-Borrego, Y. A., Soto-León, V., Gonzalez-Rosa, J. J., … Foffani, G. (2015). Safety Study of Transcranial Static Magnetic Field

Stimulation (tSMS) of the Human Cortex. *Brain Stimulation*, *8*(3), 481485. https://doi.org/10.1016/j.brs.2014.12.002

Oliviero, A., Mordillo-Mateos, L., Arias, P., Panyavin, I., Foffani, G., & Aguilar, J. (2011). Transcranial static magnetic field stimulation of the human motor cortex. *The Journal of Physiology*, *589*(Pt 20), 4949–4958. https://doi.org/10.1113/jphysiol.2011.211953

Oostenveld, R., Fries, P., Maris, E., & Schoffelen, J.-M. (2011). FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Computational Intelligence and Neuroscience*, *2011*, 1. https://doi.org/10.1155/2011/156869

Opie, G. M., Vosnakis, E., Ridding, M. C., Ziemann, U., & Semmler, J. G. (2017). Priming theta burst stimulation enhances motor cortex plasticity in young but not old adults. *Brain Stimulation*, *10*(2), 298–304. https://doi.org/10.1016/j.brs.2017.01.003

Orth, M., & Rothwell, J. C. (2004). The cortical silent period: intrinsic variability and relation to the waveform of the transcranial magnetic stimulation pulse. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *115*(5), 1076–1082. https://doi.org/10.1016/j.clinph.2003.12.025

Pani, P., Bello, F. D., Brunamonti, E., D'Andrea, V., Papazachariadis, O., & Ferraina, S. (2014). Alpha- and beta-band oscillations subserve different processes in reactive control of limb movements. *Alpha- and Beta-Band Oscillations Subserve Different Processes in Reactive Control of Limb Movements*, *8*, 383. https://doi.org/10.3389/fnbeh.2014.00383

Park, D. C., & Reuter-Lorenz, P. (2009). The adaptive brain: aging and neurocognitive scaffolding. *Annual Review of Psychology*, *60*, 173–196. https://doi.org/10.1146/annurev.psych.59.103006.093656

Pascual-Leone, A., Freitas, C., Oberman, L., Horvath, J. C., Halko, M., Eldaief, M., … Rotenberg, A. (2011). Characterizing brain cortical plasticity and network dynamics across the age-span in health and disease with TMS-EEG and TMS-fMRI. *Brain Topography*, *24*(3–4), 302–315. https://doi.org/10.1007/s10548-011-0196-8

Pascual-Leone, A., & Taylor, M. J. (2011). A developmental framework of brain and cognition from infancy to old age. *Brain Topography*, *24*(3–4), 183–186. https://doi.org/10.1007/s10548-011-0197-7

Patton, H. D., & Amassian, V. E. (1954). Single and multiple-unit analysis of cortical stage of pyramidal tract activation. *Journal of Neurophysiology*, *17*(4), 345–363.

Paulus, W., Classen, J., Cohen, L. G., Large, C. H., Di Lazzaro, V., Nitsche, M., … Ziemann, U. (2008). State of the art: Pharmacologic effects on cortical excitability measures tested by transcranial magnetic stimulation. *Brain Stimulation*, *1*(3), 151–163. https://doi.org/10.1016/j.brs.2008.06.002

Pellicciari, M., Miniussi, C., Ferrari, C., Koch, G., & Bortoletto, M. (2015). Ongoing cumulative effects of single TMS pulses on corticospinal excitability: An intra- and inter-block investigation. *Clin Neurophysiology Official J Int Fed Clin Neurophysiology*, *127*(1), 621–628. https://doi.org/10.1016/j.clinph.2015.03.002

Pitcher, J. B., Ogston, K. M., & Miles, T. S. (2003). Age and sex differences in human motor cortex input output characteristics. *The Journal of Physiology*, *546*(2), 605–613. https://doi.org/10.1113/jphysiol.2002.029454

Pogosyan, A., Gaynor, L. D., Eusebio, A., & Brown, P. (2009). Boosting cortical activity at Beta-band frequencies slows movement in humans. *Boosting Cortical Activity at Beta-Band Frequencies Slows Movement in Humans.*, *19*(19), 1637–1641. https://doi.org/10.1016/j.cub.2009.07.074

Portney, L. G., & Watkins, M. P. (2009). *Foundations of Clinical Research: Applications to Practice*. Pearson/Prentice Hall. Retrieved from https://books.google.es/books?id=apNJPgAACAAJ

Ridding, M., & Ziemann, U. (2010). Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. *J Physiology*, *588*(Pt 13), 2291–2304. https://doi.org/10.1113/jphysiol.2010.190314

Rivadulla, C., Foffani, G., & Oliviero, A. (2014). Magnetic Field Strength and Reproducibility of Neodymium Magnets Useful for Transcranial Static Magnetic Field Stimulation of the

Human Cortex. *Neuromodulation: Technology at the Neural Interface*, *17*(5), 438442. https://doi.org/10.1111/ner.12125

Rogasch, N. C., Thomson, R. H., Farzan, F., Fitzgibbon, B. M., Bailey, N. W., C, H.-P., Julio, … Fitzgerald, P. B. (2014). Removing artefacts from TMS-EEG recordings using independent component analysis: importance for assessing prefrontal and motor cortex network properties. *NeuroImage*, *101*, 425–439. https://doi.org/10.1016/j.neuroimage.2014.07.037

Rosen, A. D. (2003). Mechanism of action of moderate-intensity static magnetic fields on biological systems. *Cell Biochemistry and Biophysics*, *39*(2), 163–173. https://doi.org/10.1385/CBB:39:2:163

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., & Safety of TMS Consensus Group. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *120*(12), 2008–2039. https://doi.org/10.1016/j.clinph.2009.08.016

Rossini, P. M., Burke, D., Chen, R., Cohen, L. G., Daskalakis, Z., Iorio, R. D., … Ziemann, U. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *126*(6), 1071–1107. https://doi.org/10.1016/j.clinph.2015.02.001

Rotenberg, A., Horvath, J. C., & Pascual-Leone, A. (2014). Transcranial magnetic stimulation. https://doi.org/10.1007/978-1-4939-0879-0

Rothwell, J. . (2017). STOP: proceed No Further Without an In-depth Understanding of How Stimulation Interacts with Electrophysiology of Neurons and Circuits. Presented at the Presented at the 5th Science Factory: TMS-EEG Summer School and Workshop, Solvalla, Finland.

Ruohonen, J., & Karhu, J. (2010). Navigated transcranial magnetic stimulation. *Neurophysiologie Clinique = Clinical Neurophysiology*, *40*(1), 7–17. https://doi.org/10.1016/j.neucli.2010.01.006

Rusu, C. V. V., Murakami, M., Ziemann, U., & Triesch, J. (2014). A model of TMS-induced I-waves in motor cortex. *Brain Stimul*, *7*(3), 401–414. https://doi.org/10.1016/j.brs.2014.02.009

Sakai, K., Ugawa, Y., Terao, Y., Hanajima, R., Furubayashi, T., & Kanazawa, I. (1997). Preferential activation of different I waves by transcranial magnetic stimulation with a figure-of-eight-shaped coil. *Experimental Brain Research*, *113*(1), 24–32.

Salvador, R., Silva, S., Basser, P. J., & Miranda, P. C. (2011). Determining which mechanisms lead to activation in the motor cortex: A modeling study of transcranial magnetic stimulation using realistic stimulus waveforms and sulcal geometry. *Clinical Neurophysiology*, *122*(4), 748–758. https://doi.org/10.1016/j.clinph.2010.09.022

Sankarasubramanian, V., Roelle, S. M., Bonnett, C. E., Janini, D., Varnerin, N. M., Cunningham, D. A., … Plow, E. B. (2015). Reproducibility of transcranial magnetic stimulation metrics in the study of proximal upper limb muscles. *Journal of Electromyography and Kinesiology : Official Journal of the International Society of Electrophysiological Kinesiology*, *25*(5), 754–764. https://doi.org/10.1016/j.jelekin.2015.05.006

Schambra, H. M., Ogden, R. T., Martinez-Hernandez, I. E., Lin, X., Chang, Y. B., Rahman, A., … Krakauer, J. W. (2015). The reliability of repeated TMS measures in older adults and in patients with subacute and chronic stroke. *Frontiers in Cellular Neuroscience*, *9*, 335. https://doi.org/10.3389/fncel.2015.00335

Schilberg, L., Schuhmann, T., & Sack, A. T. (2017). Interindividual Variability and Intraindividual Reliability of Intermittent Theta Burst Stimulation-induced Neuroplasticity Mechanisms in the Healthy Brain. *Journal of Cognitive Neuroscience*, *29*(6), 1022–1032. https://doi.org/10.1162/jocn_a_01100

Schlamann, M., Yoon, M.-S., Maderwald, S., Pietrzyk, T., Bitz, A. K., Gerwig, M., … Kastrup, O. (2010). Short term effects of magnetic resonance imaging on excitability of the motor

cortex at 1.5T and 7T. *Academic Radiology*, *17*(3), 277–281. https://doi.org/10.1016/j.acra.2009.10.004

Seo, H., Schaworonkow, N., Jun, S. C., & Triesch, J. (2016). A multi-scale computational model of the effects of TMS on motor cortex. *F1000Research*, *5*. https://doi.org/10.12688/f1000research.9277.3

Shellock, F. G., & Crues, J. V. (2004). MR procedures: biologic effects, safety, and patient care. *Radiology*, *232*(3), 635–652. https://doi.org/10.1148/radiol.2323030830

Siebner, H. R., Dressnandt, J., Auer, C., & Conrad, B. (1998). Continuous intrathecal baclofen infusions induced a marked increase of the transcranially evoked silent period in a patient with generalized dystonia. *Muscle & Nerve*, *21*(9), 1209–1212.

Siebner, H. R., Lang, N., Rizzo, V., Nitsche, M. A., Paulus, W., Lemon, R. N., & Rothwell, J. C. (2004). Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(13), 3379–3385. https://doi.org/10.1523/JNEUROSCI.5316-03.2004

Silbert, B. I., Pevcic, D. D., Patterson, H. I., Windnagel, K. A., & Thickbroom, G. W. (2013). Inverse Correlation Between Resting Motor Threshold and Corticomotor Excitability After Static Magnetic Stimulation of Human Motor Cortex. *Brain Stimulation*. https://doi.org/10.1016/j.brs.2013.03.007

Silva, S., Basser, P. J., & Miranda, P. C. (2008). Elucidating the mechanisms and loci of neuronal excitation by transcranial magnetic stimulation using a finite element model of a cortical sulcus. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *119*(10), 2405–2413. https://doi.org/10.1016/j.clinph.2008.07.248

Silvanto, J., & Pascual-Leone, A. (2008). State-dependency of transcranial magnetic stimulation. *Brain Topography*, *21*(1), 1–10. https://doi.org/10.1007/s10548-008-0067-0

Sohn YH, Jung HY, Kaelin-Lang A, & Hallett M. (2002). Effect of levetiracetam on rapid motor learning in humans. *Archives of Neurology*, *59*(12), 1909–1912. https://doi.org/10.1001/archneur.59.12.1909

Sommer, M., Alfaro, A., Rummel, M., Speck, S., Lang, N., Tings, T., & Paulus, W. (2006). Half sine, monophasic and biphasic transcranial magnetic stimulation of the human motor cortex. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *117*(4), 838–844. https://doi.org/10.1016/j.clinph.2005.10.029

Sommer, M., Norden, C., Schmack, L., Rothkegel, H., Lang, N., & Paulus, W. (2013). Opposite Optimal Current Flow Directions for Induction of Neuroplasticity and Excitation Threshold in the Human Motor Cortex. *Brain Stimulation*, *6*(3), 363–370. https://doi.org/10.1016/j.brs.2012.07.003

St Pierre, T. G., & Dobson, J. (2000). Theoretical evaluation of cell membrane ion channel activation by applied magnetic fields. *European Biophysics Journal : EBJ*, *29*(6), 455–456.

Stephani, C., Paulus, W., & Sommer, M. (2016). The effect of current flow direction on motor hot spot allocation by transcranial magnetic stimulation. *Physiological Reports*, *4*(1). https://doi.org/10.14814/phy2.12666

Stokes, M. G., Barker, A. T., Dervinis, M., Verbruggen, F., Maizey, L., Adams, R. C., & Chambers, C. D. (2013). Biophysical determinants of transcranial magnetic stimulation: effects of excitability and depth of targeted area. *Biophysical Determinants of Transcranial Magnetic Stimulation: Effects of Excitability and Depth of Targeted Area*, *109*(2), 437–444. https://doi.org/10.1152/jn.00510.2012

Takahashi, M., Ni, Z., Yamashita, T., Liang, N., Sugawara, K., Yahagi, S., & Kasai, T. (2005). Differential modulations of intracortical neural circuits between two intrinsic hand muscles. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *116*(12), 2757–2764. https://doi.org/10.1016/j.clinph.2005.08.024

Teo, J., Terranova, C., Swayne, O., Greenwood, R., & Rothwell, J. (2009). Differing effects of intracortical circuits on plasticity. *Experimental Brain Research. Experimentelle Hirnforschung. Experimentation Cerebrale*, *193*(4), 555–563. https://doi.org/10.1007/s00221-008-1658-4

Thach, J. S. (1968). *A behavioral effect of intense DC electromagnetic fields*. Univ. of Texas Press, Austin.

Tharayil, J. J., Goetz, S. M., Bernabei, J. M., & Peterchev, A. V. (2017). Field Distribution of Transcranial Static Magnetic Stimulation in Realistic Human Head Model. *Neuromodulation : Journal of the International Neuromodulation Society*. https://doi.org/10.1111/ner.12699

Theysohn, J. M., Maderwald, S., Kraff, O., Moenninghoff, C., Ladd, M. E., & Ladd, S. C. (2008). Subjective acceptance of 7 Tesla MRI for human imaging. *Magma (New York, N.Y.)*, *21*(1–2), 63–72. https://doi.org/10.1007/s10334-007-0095-x

Thompson, P. D., Day, B. L., Crockard, H. A., Calder, I., Murray, N. M., Rothwell, J. C., & Marsden, C. D. (1991). Intra-operative recording of motor tract potentials at the cervico-medullary junction following scalp electrical and magnetic stimulation of the motor cortex. *Journal of Neurology, Neurosurgery & Psychiatry*, *54*(7), 618. https://doi.org/10.1136/jnnp.54.7.618

Thut, G., Schyns, P. G., & Gross, J. (2011). Entrainment of perceptually relevant brain oscillations by non-invasive rhythmic stimulation of the human brain. *Frontiers in Psychology*, *2*, 170. https://doi.org/10.3389/fpsyg.2011.00170

Tian, L.-X., Pan, Y.-X., Metzner, W., Zhang, J.-S., & Zhang, B.-F. (2015). Bats respond to very weak magnetic fields. *PloS One*, *10*(4), e0123205. https://doi.org/10.1371/journal.pone.0123205

Todd, G., Butler, J. E., Gandevia, S. C., & Taylor, J. L. (2006). Decreased input to the motor cortex increases motor cortical excitability. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *117*(11), 2496–2503. https://doi.org/10.1016/j.clinph.2006.07.303

Tremblay, S., Vernet, M., Bashir, S., Pascual-Leone, A., & Theoret, H. (2015). Theta burst stimulation to characterize changes in brain plasticity following mild traumatic brain injury: A proof-of-principle study. *Restorative Neurology and Neuroscience*, *33*(5), 611–620. https://doi.org/10.3233/RNN-140459

Triesch, J., Zrenner, C., & Ziemann, U. (2015). Modeling TMS-induced I-waves in human motor cortex. *Prog. Brain Res.*, *222*, 105–124. https://doi.org/10.1016/bs.pbr.2015.07.001

Triggs, W. J., Cros, D., Macdonell, R. A., Chiappa, K. H., Fang, J., & Day, B. J. (1993). Cortical and spinal motor excitability during the transcranial magnetic stimulation silent period in humans. *Brain Research*, *628*(1–2), 39–48. https://doi.org/10.1016/0006-8993(93)90935-G

Trippe, J., Mix, A., Aydin-Abidin, S., Funke, K., & Benali, A. (2009). theta burst and conventional low-frequency rTMS differentially affect GABAergic neurotransmission in the rat cortex. *Experimental Brain Research*, *199*(3–4), 411–421. https://doi.org/10.1007/s00221-009-1961-8

Valero-Cabré, A., Pascual-Leone, A., & Rushmore, R. J. (2008). Cumulative sessions of repetitive transcranial magnetic stimulation (rTMS) build up facilitation to subsequent TMS-mediated behavioural disruptions. *The European Journal of Neuroscience*, *27*(3), 765–774. https://doi.org/10.1111/j.1460-9568.2008.06045.x

Vallence, A.-M., Goldsworthy, M. R., Hodyl, N. A., Semmler, J. G., Pitcher, J. B., & Ridding, M. C. (2015). Inter- and intra-subject variability of motor cortex plasticity following continuous theta-burst stimulation. *Neuroscience*, *304*. https://doi.org/10.1016/j.neuroscience.2015.07.043

Valls-Solé, J. (2000). Neurophysiological characterization of parkinsonian syndromes. *Neurophysiologie Clinique/Clinical Neurophysiology*, *30*(6), 352367. https://doi.org/10.1016/S0987-7053(00)00236-7

Valls-Solé, J., Pascual-Leone, A., Wassermann, E. M., & Hallett, M. (1992). Human motor evoked responses to paired transcranial magnetic stimuli. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, *85*(6), 355–364. https://doi.org/10.1016/0168-5597(92)90048-G

van Albada, S. J., & Robinson, P. A. (2007). Transformation of arbitrary distributions to the normal distribution with application to EEG test-retest reliability. *Journal of Neuroscience Methods*, *161*(2), 205–211. https://doi.org/10.1016/j.jneumeth.2006.11.004

VanRullen, R. (2016). Perceptual Cycles. *Trends in Cognitive Sciences*, *20*(10), 723–735. https://doi.org/10.1016/j.tics.2016.07.006

Vernet, M., Bashir, S., Yoo, W.-K. K., Perez, J. M., Najib, U., & Pascual-Leone, A. (2013). Insights on the neural basis of motor plasticity induced by theta burst stimulation from TMS-EEG. *The European Journal of Neuroscience*, *37*(4), 598–606. https://doi.org/10.1111/ejn.12069

Wagner, T., Valero-Cabré, A., & Pascual-Leone, A. (2007). Noninvasive human brain stimulation. *Annual Review of Biomedical Engineering*, *9*, 527–565. https://doi.org/10.1146/annurev.bioeng.9.061206.133100

Ward, N. S., & Frackowiak, R. S. J. (2003). Age-related changes in the neural correlates of motor performance. *Brain : A Journal of Neurology*, *126*(Pt 4), 873–888.

Wassermann, E., Epstein, C., Ziemann, U., Walsh, V., Paus, T., & Lisanby, S. (2008). *Oxford handbook of transcranial stimulation*. Oxford University Press.

Werhahn, K. J., Fong, J. K., Meyer, B. U., Priori, A., Rothwell, J. C., Day, B. L., & Thompson, P. D. (1994). The effect of magnetic coil orientation on the latency of surface EMG and single motor unit responses in the first dorsal interosseous muscle. *Electroencephalography and Clinical Neurophysiology*, *93*(2), 138–146.

Werhahn, K. J., Kunesch, E., Noachtar, S., Benecke, R., & Classen, J. (1999). Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *The Journal of Physiology*, *517*(Pt 2), 591–597. https://doi.org/10.1111/j.1469-7793.1999.0591t.x

Whitham, E. M., Lewis, T., Pope, K. J., Fitzgibbon, S. P., Clark, C. R., Loveless, S., … Willoughby, J. O. (2008). Thinking activates EMG in scalp electrical recordings. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *119*(5), 1166–1175. https://doi.org/10.1016/j.clinph.2008.01.024

Whitham, E. M., Pope, K. J., Fitzgibbon, S. P., Lewis, T., Clark, C. R., Loveless, S., … Willoughby, J. O. (2007). Scalp electrical recording during paralysis: quantitative evidence that EEG frequencies above 20 Hz are contaminated by EMG. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *118*(8), 1877–1888. https://doi.org/10.1016/j.clinph.2007.04.027

Wiegel, P., Niemann, N., Rothwell, J. C., & Leukel, C. (2018). Evidence for a subcortical contribution to intracortical facilitation. *The European Journal of Neuroscience*. https://doi.org/10.1111/ejn.13934

Wischnewski, M., & Schutter, D. J. (2015). Efficacy and Time Course of Theta Burst Stimulation in Healthy Humans. *Brain Stimulation*, *8*(4), 685692. https://doi.org/10.1016/j.brs.2015.03.004

Wright, K. (2014). *Adjusting Effect Sizes in Light of Reliability Estimates*. https://doi.org/10.13140/2.1.3913.9840

Zhang, Y., Chen, Y., Bressler, S. L., & Ding, M. (2008). Response preparation and inhibition: The role of the cortical sensorimotor beta rhythm. *Response Preparation and Inhibition: The Role of the Cortical Sensorimotor Beta Rhythm*, *156*(1), 238–246. https://doi.org/10.1016/j.neuroscience.2008.06.061

Ziemann, U. (2013). Pharmaco-transcranial magnetic stimulation studies of motor excitability. *Handbook of Clinical Neurology*, *116*, 387–397. https://doi.org/10.1016/B978-0-444-53497-2.00032-2

Ziemann, U., Corwell, B., & Cohen, L. G. (1998). Modulation of plasticity in human motor cortex after forearm ischemic nerve block. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *18*(3), 1115–1123.

Ziemann, U., Reis, J., Schwenkreis, P., Rosanova, M., Strafella, A., Badawy, R., & Muller-Dahlhaus, F. (2015). TMS and drugs revisited 2014. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *126*(10), 1847–1868. https://doi.org/10.1016/j.clinph.2014.08.028

Ziemann, U., & Rothwell, J. C. (2000). I-waves in motor cortex. *Journal of Clinical Neurophysiology : Official Publication of the American Electroencephalographic Society*, *17*(4), 397–405.

Ziemann, U., Rothwell, J. C., & Ridding, M. C. (1996). Interaction between intracortical inhibition and facilitation in human motor cortex. *The Journal of Physiology*, *496*(3), 873–881. https://doi.org/10.1113/jphysiol.1996.sp021734

Ziemann, U., Tam, A., Butefisch, C., & Cohen, L. G. (2002). Dual modulating effects of amphetamine on neuronal excitability and stimulation-induced plasticity in human motor cortex. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *113*(8), 1308–1315. https://doi.org/10.1016/S1388-2457(02)00171-2

APPENDICES

APPENDIX A. Self-reported Medical History.

	Yes	No
Have you ever been hospitalized for surgery or a serious illness? If yes, please explain: _____		
Are you taking any medications (including over the counter medications such as, vitamins, cold medication, allergy medication)? If yes, please list here:		
Please list any allergies: _____		
Do you smoke? If yes, how many cigarettes/packs per day?_____		
Do you drink alcohol? If yes, how much and how often? _____		
Do you use recreational drugs? If yes, describe what drug(s) and how often: _____		
Do you drink caffeine? If yes, How much per day? _____		

Have you EVER had, or do you have any of the following? If yes, please check the box(es) below and provide any details in the comments section provided.

Heart problems		Stroke		Arthritis or joint pain	
Pacemaker		Migraines		Eczema or chronic rash	
High blood pressure		Severe headaches		Kidney disease	
Lung or breathing problems		Seizures or epilepsy		Tumor or cancer	
Asthma		Fainting or dizzy spells		Depression or anxiety	
Stomach or intestinal disease		Neck pain/injury		Psychiatric problems	
Diabetes		Eye injuries/surgeries		Drug/alcohol dependency	
Low blood sugar		Hearing loss or problems		Other:	
Liver disease		Thyroid disease			

Provide an explanation to ALL of the checked boxes above:

_____ _____

Participant Signature Date

APPENDIX B. Modified Edinburgh questionnaire.

Which of the following do you consider yourself to be?

☐ Right Handed ☐ Left Handed ☐ Ambidextrous

	Always Left	Usually Left	No Preference	Usually Right	Always Right
Writing					
Drawing					
Throwing					
Scissors					
Toothbrush					
Holding a knife to cut meat					
Spoon					
Broom (upper hand)					
Striking a match					
Opening a box					

_____ _____

Investigator Signature Date

APPENDIX C. TMS Safety Screening.

Subject Initials:	Protocol:	Date: _____/_____/_____

Check Yes or No for each question, for ALL Yes questions, provide details below	**Yes**	**No**
Do you have epilepsy or have you ever had a convulsion or a seizure?	☐	☐
Have you ever had a fainting spell (syncope)?	☐	☐
Have you ever had a head trauma that was diagnosed as a concussion or was associated with a loss of consciousness?	☐	☐
Do you have any hearing problems or ringing in your ears?	☐	☐
Do you have cochlear implants?	☐	☐
Are you pregnant or any chance that you might be?	☐	☐
Do you have any metal in the brain, skull or elsewhere in your body? (e.g. splinters, fragments, clips, etc.…). If yes, ***specify the type of metal***.	☐	☐
Do you have a neurostimulator in your body (e.g. vagal nerve stimulator, deep brain stimulator, epidural/subdural stimulator)?	☐	☐
Do you have a cardiac pacemaker or intracardiac lines?	☐	☐
Do you have a medication infusion device?	☐	☐
Did you ever undergo TMS in the past? If yes, were there any problems (***describe below***)?	☐	☐
Did you ever undergo an MRI in the past? If yes, were there any problems (***describe below***)?	☐	☐
Are you taking any medications (including over the counter medications)? If yes, ***then please list below***.	☐	☐

For any YES response, please provide details below. Please list ALL medications and please list any past neurological (relating to your brain or spinal cord) medical or surgical history.

_____

_____

Investigator Signature:	Date: _____/_____/_____
Subject Signature:	Date: _____/_____/_____

APPENDIX D. tSMS safety screening.

tSMS Safety Screening Subject ID:_____
Date:_____ Visit #____

Check Yes or No for each question, for ALL Yes questions, provide details below	Yes	No
Have you ever been a machinist, welder or metal worker? If yes, did you wear safety glasses at all times?	□	□
Have you ever had a piece of metal in your eyes?	□	□
Do you have a Cardiac Pacemaker?	□	□
Do you have an Aneurysm clip?	□	□
Do you have a Prosthetic Heart Valve?	□	□
Do you have a Neurostimulator (Tens-Unit) or any implanted pumps (Insulin)?	□	□
Do you have Cochlear implants?	□	□
Do you have a Stainless-Steel IUD?	□	□
Do you have any shrapnel in your body?	□	□
Do you have any metal or metallic implants in your body that are not listed?	□	□
Is there a chance you could be pregnant?	□	□
Are you wearing a skin patch (Nicotine, contraceptive)?	□	□
Have you had a bone treated with metal rods, plates, or screws? If yes, please describe and include date(s)_____ _____	□	□
Have you had any major surgery? If yes, please describe and include date(s):_____ _____	□	□
Do you have any removable dental work?	□	□
Do you have a hearing aid?	□	□
Do you have tattoos?	□	□
Do you have piercings that have not been removed?	□	□

For any YES response, please provide details below:

_____
_____
_____
_____

YOU WILL BE ASKED TO LEAVE THESE ITEMS IN A BOX AT A SAFE DISTANCE OF THE MAGNET:

Hairpins/Barrettes/Safety Pins
Wigs/Hairpiece/Extensions
Jewelry/Piercings
Watch

Any other removable metal objects not listed that are above your shoulders or on your arms

Subject Signature_____ Date_____

Investigator signature_____ Date_____

APPENDIX E. Side Effects Questionnaire.

Subject Initials/Number:	Date:	Visit:	Protocol :
Check Appropriately: □ rTMS □ Single pulse TMS □ Paired pulse TMS			

Do you have any of the following symptoms?										
Symptoms	Yes/No If "yes" to any symptoms, fill out severity and relationship		Severity 1 = Absent 2 = Mild 3 = Moderate 4 = Severe			Relationship 1 = None 2 = Remote 3 = Possible 4 = Remote 5 = Definitive			Comments
	Pre	Post	Pre		Post	Pre		Post	
Headache									
Neck Pain									
Scalp Pain/Irritation									
Only ask the following Questions Post-TMS									
Are you having any trouble hearing compared to when you arrived?									
Are you having any trouble with your thinking compared to when you arrived?									
Are you having any trouble concentrating compared to when you arrived?									
Do you have any change in your mood (positive or negative)?									
Is there anything else that you would like to tell me?									
Did the subject have a syncopal event or vertigo sensations, a seizure or any other adverse effect during or post-TMS or tSMS? **Yes No if YES then write a summary of the event**									
Any other comments (ask about phosphenes and metallic taste related to tSMS)?									

APPENDIX F. Resumen de la Tesis Doctoral

Introducción

Durante las últimas décadas, se ha generado un gran interés científico y clínico en el uso de los campos magnéticos y eléctricos como herramientas de estimulación cerebral no invasiva (Non Invasive Brain Stimulation, NIBS). Estas técnicas permiten explorar y modificar la excitabilidad y plasticidad cerebrales, así como modular redes neurales sin la necesidad de procedimientos invasivos o quirúrgicos. Por lo tanto, no es sorprendente que su uso para fines médicos haya experimentado un crecimiento exponencial y que hoy en día muchas clínicas y laboratorios incorporen métodos de NIBS como herramientas diagnósticas, terapéuticas o de investigación. Las técnicas de NIBS son, por lo tanto, un grupo heterogéneo de herramientas que usan corrientes eléctricas y/o fuerzas magnéticas para evaluar y modular las funciones del cerebro. Ejemplos destacables de estas técnicas no invasivas son la estimulación por corriente continua transcraneal (Transcranial Direct Current Stimulation, tDCS), que utiliza corrientes eléctricas para modular el sistema nervioso central (SNC); o la estimulación magnética transcraneal (Transcraneal Magnetic Stimulation, TMS), donde los campos magnéticos son capaces de modular y estimular las estructuras del sistema nervioso.

Así mismo, la combinación de técnicas de NIBS con herramientas habituales en los registros neurofisiológicos como la electromiografía (EMG), la electroencefalografía (EEG) o la resonancia magnética (MRI) permite evaluar los cambios en el sistema nervioso de una manera objetiva y cuantificable.

En esta tesis doctoral tiene gran relevancia la TMS, que desde que fue descrita por primera vez hace más de 30 años, se ha convertido en una poderosa herramienta diagnóstica y de tratamiento. La TMS se sirve de las propiedades de los campos electromagnéticos enunciadas en el s. XIX por Faraday. Es decir, un pulso de TMS consiste en una corriente eléctrica que pasa rápidamente por una bobina creando un campo magnético asociado. Este campo magnético

traspasa las barreras naturales de piel y hueso hasta llegar a la corteza cerebral. En la corteza cerebral, y dado que es un tejido eléctrico, los campos magnéticos inducen una segunda corriente eléctrica que es capaz de activar una zona determinada de esa corteza. De ese modo la TMS se ha podido utilizar para investigar la excitabilidad y reactividad corticales dentro de un área concreta o en su relación con otras áreas, para estudiar el comportamiento cerebral, evaluar la neurofisiología del cerebro sano y la patofisiología de diferentes trastornos neuropsiquiátricos. Además, cuando se aplica como un tren de pulsos repetidos con una cierta frecuencia, denominada TMS repetitiva (rTMS), modula la actividad de las redes neuronales más allá del tiempo de estimulación. El tratamiento con rTMS de la depresión resistente a fármacos es el ejemplo paradigmático del uso médico de las técnicas de NIBS. Este uso fue aprobado por la agencia americana responsable de la regulación de medicamentos (FDA) (registro de aprobación de la FDA K061053) en 2008 y desde entonces ha sido ampliamente utilizado en todo el mundo. Además, la FDA también ha aprobado más recientemente el uso de TMS para el mapeo del sistema motor y del lenguaje como herramientas de diagnóstico preoperatorio. Desde entonces, se han descrito muchos otros protocolos y aplicaciones de TMS para diferentes enfermedades neuropsiquiátricas.

Como hemos comentado previamente, las técnicas más comúnmente utilizadas se han clasificado clásicamente en dos grupos según el tipo de campo que emplean para alcanzar la corteza cerebral: (1) técnicas que usan campos electromagnéticos para pasar por el cuero cabelludo sin dolor y llegar a la corteza cerebral, como la TMS; y (2) técnicas que usan campos eléctricos, como la tDCS. Recientemente, se ha descrito en humanos sanos un método novedoso que no puede clasificarse en ninguno de los anteriores grupos, la estimulación magnética estática transcraneal (Transcraneal Static Magnetic Stimulation, tSMS). Esta nueva técnica de NIBS utiliza campos magnéticos estáticos (Static magnetic fields, SMF). El uso de SMF implica que el campo magnético no varia a lo largo del tiempo y esto se traduce en que no existe una inducción de corriente eléctrica secundaria como en la TMS. Aún así, la tSMS es capaz de modificar la

reactividad y excitabilidad de la corteza cerebral de forma indolora, reversible y segura. Los mecanismos mediante los cuales los SMF modifican la reactividad y excitabilidad corticales aún no se han dilucidado claramente. Diferentes estudios a nivel celular han propuesto varias hipótesis a este respecto: (1) debido a las características diamagnéticas y anisotrópicas de los fosfolípidos, los canales iónicos de iones y el flujo de calcio de la membrana se ven alterados bajo la influencia de los SMFs; (2) podrían también deberse un efecto de las partículas ferromagnéticas presentes en el cerebro sobre los canales iónicos; (3) o por ultimo, los SMFs podrían modificar la excitabilidad de membrana debido al efecto Hall sobre canales voltaje-dependientes, aunque esta última hipótesis ha generado mayor controversia.

A pesar del gran interés generado, todavía falta por comprender de manera profunda los mecanismos subyacentes a las técnicas NIBS y su interacción con los elementos neurales. Esto es especialmente acuciante en las nuevas formas de modulación del cerebro como la tSMS. Aunque la investigación en torno a la tSMS se está desarrollando rápidamente, aún quedan muchas preguntas por responder para entender cómo interactúan los SMF con la corteza cerebral. Por otra parte, el hallazgo de cambios significativos después de una intervención requiere evaluaciones neurofisiológicas fiables y reproducibles. Varios factores pueden influir en la respuesta a los métodos de NIBS como TMS, lo que reduce su reproducibilidad y afecta los posibles resultados.

El objetivo principal de esta tesis es profundizar en el conocimiento de la interacción de las técnicas de NIBS con los componentes corticales respondiendo dos preguntas principales.

En primer lugar, identificar y comprender mejor los factores que potencialmente influyan en los efectos y la reproducibilidad de la TMS. A pesar de su relevancia, la TMS tiene una considerable variabilidad entre visitas o dependiendo de la persona que suministra la estimulación. La determinación de diferentes elementos que estén aportando variabilidad puede ayudar a mejorar la reproducibilidad y, por lo tanto, la utilidad de la técnica para fines diagnósticos y terapéuticos. Para responder a esta pregunta se investigaron dos tipos de factores. En primer

lugar, factores técnicos o modificables. En base a los modelos teóricos actuales, diferentes parámetros técnicos, por ejemplo, diferentes formas de onda o direcciones de corriente de los pulsos de TMS, activan diferentes redes neurales en el córtex. Por lo tanto, el primer experimento de esta tesis plantea la primera hipótesis de que la forma de onda y la dirección de corriente de la TMS desempeñan un papel importante en la reproducibilidad al interactuar con componentes neurales específicos. El segundo grupo de factores que se investigaron en este trabajo fueron factores que no son fácilmente modificables y que afectan a la reactividad y a los procesos de plasticidad cerebrales. En concreto en estos experimentos se incluyeron adultos mayores de 55 años y pacientes con alteraciones metabólicas (Diabetes Mellitus tipo 2, T2DM) y cognitivas (Demencia por enfermedad de Alzheimer, AD) para observar los efectos de la edad y de las enfermedades relacionadas con la edad en la reproducibilidad de la TMS. La hipótesis de este estudio fue que estos factores fisiológicos y patológicos modifican la interacción TMS-córtex cerebral, así como también tendrán un impacto considerable en la fiabilidad de TMS.

En la segunda parte de esta tesis, se intenta investigar más a fondo el comportamiento y los cambios en la excitabilidad del córtex motor tras la exposición a la tSMS. Para registrar los cambios en la reactividad cerebral producidos por la tSMS utilizamos las respuestas motoras (motor evoked potentials, MEP) a la TMS y los registros EEG como herramientas de evaluación neurofisiológica. El EEG recoge los cambios eléctricos de la convexidad del cerebro a lo largo del tiempo de una manera muy precisa. Por lo tanto, los registros de EEG junto con las evaluaciones motoras de la TMS pueden ayudarnos a comprender mejor los cambios fisiológicos debido a tSMS. Como se mencionó anteriormente, parámetros físicos específicos de la TMS pueden interactuar con diferentes células neurales. En base a esto, en este tercer experimento de la tesis, se evaluaron los efectos de la tSMS sobre el córtex motor con varias formas de onda y direcciones de corriente. La hipótesis principal de trabajo fue que la tSMS influye de manera específica en diferentes redes neurales y por lo tanto las respuestas EMG serán distintivas dependiendo de los parámetros de TMS. Para obtener más evidencia, se llevaron a cabo no solo

protocolos de TMS de pulsos simples (excitabilidad cortical), si no también la evaluación del equilibrio excitación / inhibición en el córtex motor mediante TMS de pulsos-pareados. Los protocolos de pulsos pareados nos ayudaron a comprender mejor el funcionamiento de los circuitos intracorticales inhibitorios y facilitadores. Por último, los registro EEG y su relación con las respuestas EMG a la TMS aportaron mayor evidencia a las tesis planteadas.

Estudios de reproducibilidad

Previamente se ha mencionado que los estudios de reproducibilidad de esta tesis se pueden dividir en dos teniendo en cuenta los factores que se estudiaron.

Por una parte, en un primer estudio se evaluó la influencia de los parámetros técnicos, es decir la forma de onda y dirección de corriente de la TMS.

En este estudio se recogieron los datos en dos visitas diferentes en 23 sujetos jóvenes y sanos. Se usaron para ello tres formas de onda y direcciones de corriente: (1) monofásico posterior-anterior ($mono_{PA}$) (9 sujetos), (2) monofásico anterior-posterior ($mono_{AP}$) (7 sujetos) y (3) bifásico anterior-posterior—posterior-anterior (Bi_{AP-PA}) (7 sujetos).

Cada visita consistió en una serie de evaluaciones neurofisiológicas realizadas con TMS con neuronavegación. Por una parte, se evaluó la excitabilidad y reactividad cortical con pulsos simples. Los protocolos de pulsos simples incluyeron la intensidad mínima a la cual existe respuesta motora o umbral motor (resting motor threshold, RMT), la amplitud y latencia de la respuesta corticoespinal a pulsos supra umbral o MEP y la duración del periodo de silencio (cortical silent period, cSP). Se exploró también el equilibrio inhibición / facilitación mediante una serie de protocolos de pulsos pareados inhibitorios (inhibición corta (SICI) o larga (LICI)) y facilitadores (ICF).

En un segundo experimento de reproducibilidad, se investigó la influencia de factores fisiológicos de la edad y patofisiológicos de enfermedades asociadas a la edad (es decir, AD y T2DM) en la fiabilidad de los protocolos de pulsos simples y pareados de TMS, así como de un

tipo específico de rTMS que se denomina estimulación por theta-burst intermitente (intermittent theta-burst stimulation, iTBS). Para ello se obtuvieron datos de 36 adultos de los cuales 9 padecían AD, 15 T2DM y los 12 restantes eran sujetos adultos sanos de edades similares a los otros dos grupos. Se obtuvo también un perfil genético de los participantes para saber cuáles de ellos eran portadores de la variante Met en el polimorfismo del factor neurotrófico derivado del cerebro (Brain derived neurotrophic factor, BDNF).

En ambos experimentos de reproducibilidad nos centramos en el estudio de la fiabilidad y consistencia de respuesta intra-sujeto. Para ello se utilizó el método estadístico que se ha descrito como el más adecuado, los coeficientes de correlación intraclase (Intraclass correlation coefficients, ICC). Este coeficiente cuantifica cuánta de la variabilidad observada de una respuesta pertenece a la heterogeneidad de la muestra y cuánta a la variabilidad a las diferentes visitas o diferentes evaluadores. Por lo tanto, valores ICC de 1 reflejan la máxima fiabilidad de una medida, y 0 indica que la fiabilidad es muy pobre o no existe.

En base a los resultados de la presente tesis, se pueden extraer varias conclusiones generales. De ambos estudios podemos concluir que los umbrales motores siguen siendo la medida más fiable independientemente de la forma de onda, la dirección de la corriente, la edad o grupo de enfermedad, dado que sus ICC son los más altos y cercanos a 1.

Sin embargo, los parámetros técnicos de la TMS estudiados en esta tesis sí influyen en la eficacia y la fiabilidad de los protocolos de pulso simples y pareados. De especial relevancia son los efectos sobre el RMT ya que generalmente este se toma como referencia para calcular la intensidad del resto de los protocolos. Además, en ambos experimentos, se observó una correlación negativa entre el RMT y la amplitud de los MEPs. A su vez, esta amplitud de MEP se asoció de manera inversa con la iTBS.

De manera novedosa, en esta tesis los protocolos de pulsos pareados se realizaron con formas de onda y direcciones de corriente que no se habían usado previamente en publicaciones anteriores ($mono_{AP}$ y bi_{AP-PA}) además de con $mono_{PA}$ que es el parámetro de uso habitual. Estas

formas de onda y direcciones de corriente influyeron en los efectos y la fiabilidad de TMS de una manera característica. En particular, las formas de onda monofásicas lograron una inhibición mayor y más reproducible, y las formas de onda con el componente AP alcanzaron una mayor facilitación. Es más, la bi_{AP-PA} podría ser la forma de onda de elección para evaluar los efectos de la TMS en protocolos como cSP o ICF en los que podrían tener gran relevancia las conexiones córtico-corticales o la interacción de más de una estructura cerebral.

Los procesos fisiológicos de envejecimiento y la fisiopatología de las enfermedades relacionadas con la edad pueden modificar la fiabilidad de TMS. En este trabajo de tesis doctoral, esto se observó en dos de los diferentes análisis.

Primero, utilizando $mono_{PA}$ se observaron ICC más altos tanto en RMT como en LICI en la comparación entre adultos jóvenes y mayores. Aunque algunas características de la metodología probablemente hayan podido contribuir a estas diferencias, la mayor rigidez fisiológica del cerebro según avanza la edad podría afectar a la habilidad del SNC de variar entre pulsos, por lo que los valores de ICC serán más altos.

La mayor rigidez de los procesos cerebrales también ha desempeñado un papel importante para el grupo de AD. El grupo de AD mostró una mayor fiabilidad en casi todas las medidas de TMS, en particular aquellas después de iTBS, comparado con los adultos mayores o los T2DM. Por el contrario, los otros dos grupos mostraron una fiabilidad bastante baja de los efectos de iTBS. Finalmente, otros factores también pueden contribuir a aumentar la variabilidad de las respuestas a TMS y, en particular, a iTBS. Entre ellos, dos fueron relevantes para este trabajo porque se comprobó que aumentaron la variabilidad de las respuestas en nuestra muestra: (1) Portadores de la variante Met del polimorfismo BDNF, (2) y los días entre ambas visitas. Los intervalos entre visitas de menos de una semana aumentaron significativamente la variabilidad en las respuestas a la iTBS, probablemente reflejando procesos de metaplasticidad. Sin embargo, estos dos factores no parecen tener influencia en las otras medidas de pulsos simples y pareados.

Estudios sobre campos magnéticos estáticos

Los campos magnéticos y eléctricos dinámicos se han utilizado durante décadas para explorar la función del cerebro humano, la fisiología del cerebro en la salud y la enfermedad, y se ha demostrado que modulan la actividad del cerebro ayudando en el tratamiento de diferentes enfermedades. El ejemplo más conocido de esto es TMS. Recientemente, varios estudios han encontrado que los SMF moderados (es decir, campos magnéticos entre 1mT y 1T que no cambian con el tiempo) también influyen en la excitabilidad cortical humana.

Hoy en día sabemos que la exposición a tSMS durante 10-15 min induce una reducción de la amplitud de los MEPs de aproximadamente el 25% que dura más que la intervención y que además se correlaciona negativamente con un aumento en el RMT. Esto traduce una disminución de la excitabilidad cortical motora debido a los efectos de la SMF. Tras estos estudios iniciales, otros grupos de investigación continuaron explorando los efectos de tSMS en (1) corteza motora, realizando diferentes protocolos inhibidores de TMS y (2) otras áreas corticales, como las áreas somatosensorial y visual.

Los estudios previos en córtex motor llegaron a la conclusión de que la tSMS reduce la excitabilidad cortical probablemente a través de circuitos intracorticales inhibidores de $GABA_A$ ya que se ha observado una disminución en SICI. Sin embargo, la participación de los circuitos inhibidores de $GABA_A$ parece no poder ser la única explicación, ya que otros protocolos que también traducen procesos mediados por $GABA_A$, como SAI o LAI, no sufrieron modificaciones tras la tSMS. Por lo tanto, los mecanismos fisiológicos de la inhibición inducida de la corteza motora después de la tSMS son todavía ampliamente desconocidos lo que hace necesarios estudios que profundicen en posibles circuitos corticales que están involucrados.

Una forma de profundizar en la comprensión de los mecanismos corticales de tSMS es mediante el uso de diferentes formas de onda TMS y direcciones de corriente. Como previamente se ha comentado en varias ocasiones, los diferentes parámetros de TMS actúan de manera

específica sobre circuitos neurales del sistema motor. Por tanto, el primer objetivo de este experimento fue ahondar en la comprensión de las interacciones tSMS-córtex motor utilizando diferentes formas de onda y direcciones corriente. Estos parámetros específicos de la TMS se usaron para realizar protocolos comunes de evaluación de la excitabilidad cortical (amplitud de MEPs) y del equilibrio entre las redes corticales facilitadoras e inhibidoras (cSP y pulsos pareados). El segundo objetivo investigó más a fondo los efectos de tSMS en el sistema motor midiendo la actividad oscilatoria cortical espontánea con EEG y relacionó la reducción en la amplitud MEP con los posibles cambios en los registros EEG. Hasta la fecha no se ha publicado ningún trabajo sobre los efectos de la tSMS en los registros de la actividad oscilatoria del córtex motor. Aún así, la bibliografía existente describe que ante la inhibición espontánea o inducida del córtex motor existe un aumento de las frecuencias del rango beta. Por lo tanto, cabría esperar que los efectos inhibitorios de la tSMS (reducción del 25% en la amplitud de los MEPs según los estudios previos) se tradujeran a su vez en un aumento de esas frecuencias beta.

En todos los sujetos de estos experimentos se llevaron a cabo dos visitas, una con estimulación tSMS real y otra placebo. El orden de ambas fue distribuido de manera aleatoria para cada sujeto. Antes y después de la intervención con tSMS se realizaron los registros de EMG y de EEG.

En primer lugar, nuestro resultados reprodujeron con bastante precisión los resultados de estudios previos en los que se mostró una disminución en la amplitud de los MEPs, un aumento en la inhibición de SICI y la tendencia del cSP a duraciones más largas. Además, hemos ampliado esos resultados con el estudio del equilibrio inhibición / facilitación mediante la realización de otros protocolos de pulsos pareados que no habían sido investigados con anterioridad. Gracias a la realización de estos pulsos pareados se observó que, tras la visita de tSMS real, se registró una mayor inhibición tras LICI y una tendencia hacia una menor facilitación o ICF.

El segundo de los hallazgos novedosos proviene del uso de diferentes parámetros de TMS. Este uso nos permitió profundizar en los efectos de los SMF en diferentes redes neuronales corticales y mostrar que la influencia de esta nueva técnica NIBS está restringida a determinados circuitos intracorticales. Más precisamente, los efectos inhibitorios de la tSMS sobre la excitabilidad cortical (amplitud MEP) y el equilibrio intracortical (protocolos de pulsos pareados y cSP) únicamente fueron revelados por la forma de onda $mono_{PA}$.

Finalmente, en la actividad oscilatoria espontánea se registró un aumento de las frecuencias del rango beta tras la tSMS real independientemente de la forma de onda o de la dirección corriente de la TMS, tal y como se había teorizado. Fortaleciendo la hipótesis de que los procesos inhibitorios son producto de la influencia de tSMS en redes neurales específicas del córtex motor, se observó que este aumento de beta en regiones fronto-centrales estaba inversamente relacionado con la disminución de amplitud de los MEPs captada por $mono_{PA}$.

Conclusiones

Los resultados de la presente tesis pueden ayudar a elegir los parámetros de los pulsos de la TMS que maximicen los efectos y la fiabilidad de las medidas realizadas. Los resultados también aportan diferentes recomendaciones para optimizar dicha reproducibilidad en poblaciones envejecidas o que sufren patologías asociadas a la edad.

Por último, en esta tesis se ha conseguido profundizar e identificar posibles mecanismos por los cuales los SMFs inducen una reducción de la excitabilidad. Sin embargo, dada la naturaleza de los estudios realizados dichos mecanismos han de ser confirmados por futuros estudios fisiológicos.

PUBLICATIONS AND CONTRIBUTIONS TO CONFERENCES

Publications

P1. *The effects of waveform and current direction on the efficacy and test-retest reliability of transcranial magnetic stimulation.* Davila-Pérez P, Jannati A, Fried PJ, Cudeiro J, Pascual-Leone A. (2018) *Neuroscience*, *393*, 97-109. https://doi.org/10.1016/j.neuroscience.2018.09.044

P2. *EEG Microstate Correlates of Fluid Intelligence and Response to Cognitive Training.* Santarnecchi E., Khanna A.R., Musaeus C.S., Benwell C.S., Davila P., Farzan F., Matham S., Pascual-Leone A., Shafi M. (2017). *Brain topography*, *30*(4), 502-520. https://doi.org/10.1007/s10548-017-0565-z

P3. *Reproducibility of Single-Pulse, Paired-Pulse, and Intermittent Theta-Burst TMS Measures in Healthy Aging, Type-2 Diabetes, and Alzheimer's Disease*. Fried P.J., Jannati A., Davila-Pérez P., Pascual-Leone A. (2017). *Frontiers in aging neuroscience*, *9*, 263. https://doi.org/10.3389/fnagi.2017.00263

P4. *Cognitively relevant neural oscillations in healthy versus pathological aging.* Benwell C.*, Davila-Pérez P.*, Fried P.J.*, Jones R.N., Travison T.G., Santarnecchi E., Pascual-Leone A., Shafi M.M. *Neurobiology of Aging* (Under review) (*Co-first authors)

P5. *Repetitive Transcranial Magnetic Stimulation in Spinocerebellar Ataxia: A Pilot Randomized Controlled Trial.* Manor B., Greenstein P.E., Davila-Perez P., Seth Wakefield S., Zhou J., Pascual-Leone A. *Frontiers Neurology* (Under review)

P6. *Grater aftereffects of intermittent theta-burst stimulation in a non-target muscle.* Morris T.P., Davila-Perez P., Jannati A., Menardi A., Pascual-Leone A., Fried P.J. *Brain Stimulation* (Under review)

P7. *Factores que influyen en la estimulación magnética transcraneal y su reproducibilidad.* Davila-Pérez P., Cudeiro J., Pascual-Leone A. *Chapter* (Under review)

P8. *A Practical Manual for Transcranial Magnetic Stimulation*. Edwards D.J., Fried P.J., Davila-Pérez P., Horvath J.C., Rotenberg A., Pascual-Leone A. *Manual* (Under review)

P9. The effects of Static Magnetic Fields on motor excitability measured with different TMS waveforms and current directions. Davila-Pérez P., Pascual-Leone A., Cudeiro J. (In preparation)

P10. The effects of Static Magnetic Fields on motor oscillatory activity. Davila-Pérez P., Musaeus C.S., Pascual-Leone A., Shafi M.M., Cudeiro J. (In preparation)

Contributions to conferences

C1. *Specificity of theta burst. November 15, 2017 Poster*
By Morris T.P., Davila-Perez P., Jannati A., Pascual-Leone A., Fried P.J.
Neuroscience 2017, the Annual Meeting of the Society for Neuroscience, Washington, DC

C2. *Reproducibility of intermittent theta-burst, paired-pulse, and single-pulse transcranial magnetic stimulation measures in older clinical populations. March 7, 2017 Poster*
by Fried P.J., Jannati A., Davila-Pérez P., Pascual-Leone A.
2nd International Brain Stimulation Conference 2017, Barcelona, Spain
http://dx.doi.org/10.1016/j.brs.2017.01.243

C3. *Transcranial magnetic stimulation as a neurophysiological biomarker in spinocerebellar ataxia. March 7, 2017 Poster*
by Davila-Pérez P.*, Jannati A.*, Wakefield S., Manor B., Greenstein P., Pascual-Leone A. (* Co-first authors)
2nd International Brain Stimulation Conference 2017, Barcelona, Spain
http://dx.doi.org/10.1016/j.brs.2017.01.240

C4. *Relationships across, alpha/beta EEG power, learning and memory, and TMS-based neurophysiology measures in persons with Alzheimer's disease, Type-2 diabetes, and controls. March 6, 2017 Poster*
by Benwell C.*, Davila-Pérez P.*, Fried P.J., Shafi M., Pascual-Leone A. (* Co-first authors)
2nd International Brain Stimulation Conference 2017, Barcelona, Spain
http://dx.doi.org/10.1016/j.brs.2017.01.407

C5. *Higher resting motor threshold associated with better cognitive function in patients with mild Alzheimer's disease. November 14, 2016 Poster*
by Fried P.J., Jannati A., Davila-Perez P., Chen V.M., Press D.Z., Pascual-Leone A.
Neuroscience 2016, the Annual Meeting of the Society for Neuroscience, San Diego, CA

C6. *The effects of waveform and current direction on test-retest reliability of transcranial magnetic stimulation. November 13, 2016 Poster*
by Davila-Perez P., Jannati A., Shafi M., Cudeiro J., Pascual-Leone A.
Neuroscience 2016, the Annual Meeting of the Society for Neuroscience, San Diego, CA

C7. *Standardized method for training and assessing competency in the application of transcranial magnetic stimulation*. *November 12, 2016 Poster*
by Fried P.J., Davila Perez P., Jannati A., Pascual-Leone A.
Neuroscience 2016, the Annual Meeting of the Society for Neuroscience, San Diego, CA

C8. *Tratamiento del dolor crónico mediante técnicas de estimulación no invasiva. June 2016 Invited Communication*
XIII Congreso de la Sociedad Española del Dolor, Pamplona.

C9. *Neurophysiological correlates of cognitive dysfunction in Alzheimer's disease and Type-2 diabetes. 2016 Poster*
by Fried P.J., Davila-Perez P., Cypess A.M., Pascual-Leone A.
29th Annual Scientific Symposium, Massachusetts Alzheimer's Disease Research Center, Boston University Alzheimer's Disease Center and Harvard NeuroDiscovery Center.

C10. *TMS in Spinocerebellar Ataxia. 2015 Poster*
by Davila Perez P., Jannati A., Wakefield S., Manor B., Greenstein P., Pascual-Leone A.
3rd Science Factory: TMS–EEG Summer School. Aalto University, Helsinki.